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Diversity and community structure of Ephemeroptera in freshwater stream of Megamalai hills, Tamil Nadu, India

S. Barathy*, T. Sivaruban#, Srinivasan Pandiarajan, Isack Rajasekaran and M. Bernath Rosi

Department of Zoology, Fatima College, Madurai-625018, Tamil Nadu, India; #PG and Research Department of Zoology, The American College (Autonomous), Madurai-625002, Tamil Nadu, India.
Email: barathyruban@gmail.com

ABSTRACT: In the study on the diversity and community structure of Ephemeroptera in the freshwater stream of Chinnasuruli falls on Megamalai hills, a total of 523 specimens belonging to thirteen genera and five families were collected in six month periods. Of the five families, Teloganodidae and Leptophlebiidae exhibited high diversity and Caenidae showed low diversity. *Choroterpes alagarensis* (Leptophlebiidae) is the most dominant species. Diversity indices such as Shannon and Simpson indices showed that diversity was maximum in November and December and it was minimum in August and January. Canonical Correspondence Analysis revealed that rainfall, water flow, turbidity, and air temperature were the major stressors in affecting the Ephemeropteran community structure.

KEYWORDS: Mayflies, Shannon and Simpson indices, Canonical Correspondence Analysis

INTRODUCTION

Order Ephemeroptera commonly known as mayflies have larval stages in the aquatic environment and they inhabit the freshwater ecosystem. Mayfly nymphs mainly form the main part of the freshwater ecosystem. They change their abundance and diversity when habitats change or become depleted and are called bio-indicators of good water quality (Barathy *et al.,* 2020a). Along with Trichoptera and Plecoptera, mayflies are excellent biological indicators of water quality, due to their high level of sensitivity to pollution and anthropogenic effects (Rosenberg and Resh, 1993). Likewise, they play an important role in the nutrient cycle by degrading large amounts of organic matter in aquatic habitats, shaping an important part of the food chain and food web in freshwater habitats.

In recent years, numerous works were done in the taxonomic aspects and least known families in India like Tricorythidae, Teloganodidae, and Caenidae have been revealed (Sivaruban *et al.,* 2021; Srinivasan *et al.,* 2021a; Srinivasan *et al.,* 2021b) and some ecological works were carried out in both Western and Eastern Ghats of southern India (Barathy*et al.,* 2020b; Sivaruban *et al.,* 2020b; Srinivasan *et al.,* 2019) yet it stays fragmentary. As one of the biodiversity hotspots in the world, the Western Ghats occupy a variety of ecological niches and are unique in their ecological structure and functions. Megamalai is one of the high

* Author for correspondence

altitudinal places in Tamil Nadu and no studies were made formally in both taxonomy and ecological aspects in this eco-region. Therefore, the present study aims to evaluate the diversity and distribution of mayflies in correlation with physicochemical variables of water, as well as to provide information on the inventory of mayflies in the Megamalai region of Western Ghats.

MATERIALS AND METHODS

Chinnasuruli falls also known as "Cloud Land" falls is situated near Kombaithozhu Village of the Theni district of Southern Western Ghats of Tamil Nadu, India. The waterfalls originate from the Megamalai hills. The waterfalls cascade from the height of 190 feet. Coordinates: latitude 9°42'30" N and longitude 77°25'34" E. The present work was undertaken from August 2017 to January 2018. Water samples were collected from the stream and they were analyzed using APHA guidelines (APHA, 2005). Collection of benthic specimens was done using a 1m wide Kick-net (Burton and Sivaramakrishnan, 1993) with a mesh size of approximately 1 mm. All insects were picked and preserved in 80 percent ethyl alcohol. Collected samples were placed under a stereomicroscope (Magnus Pro) and identified with the help of field guides by Sivaramakrishnan *et al.* (1998) and Barathy *et al.* (2021).

Physico-chemical parameters of Chinnasuruli stream, water temperature (°C), air temperature (°C), water flow (m\s), water pH, dissolved oxygen (mg/l), total dissolved solids (ppt), turbidity (NTU), and mean monthly rainfall (mm) were taken for the period August – January.

The data analysis was done with the help of the PAST software (Version 4.2) to measure various diversity indices and Canonical Correspondence Analysis (CCA) was also analyzed (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Sampling of Ephemeropteran larvae resulted in a total of 523 specimens belonging to 13 genera and five families (Table 1). Of the five families, Teloganodidae and Leptophlebiidae exhibited high diversity which includes five and four species respectively and encompassing 145 and 162 individuals respectively. On the other hand, Caenidae exhibits low diversity with only two species and encompassing 30 individuals. On the whole, *Choroterpes alagarensis* Dinakaran, Balachandran & Anbalagan, 2009 (Leptophlebiidae) is the most dominant species and it shows a wide range of tolerance to the physicochemical variables.

When diversity richness measured based on months indicated higher richness in October and November compared to other months. The richness was comparatively very low in these months and it is probably due to high temperature and low rainfall. Among alpha diversity indices, the Shannon-Weiner index and Simpson's index calculated to show that the Shannon index was higher in December (2.637) and lowest in August (2.415), while Simpson index was higher in November (0.9211) and lowest in August (0.8959). Results reveal that high rainfall months support more diverse taxa in contrast with non-rainy periods like August and January (Table 2). Similar results were observed by Sivaruban *et al.* (2020a) in the Gadana River.

Both water temperature and air temperature were higher in August and January compared to other months (Table 3). This also leads to a decline of low tolerant taxa in these months and supports high tolerant taxa like *Caenis* sp. The water flow velocity in Chinnasuruli stream during August was found to be 0.50 m/s which was low compared to other months and it lead to a decline of the rithral mayflies like *Epeorus* in August. Dissolved oxygen (DO) level stays normal in all the months and pH values stay similar in all months except there is a slight variation in August and January showing the water is slightly alkaline but falls within the normal range. Turbidity becomes a more vital component next to rainfall in this stream, as more turbid water was seen in August (0.30 NTU), this is due to low water flow and this subsequently leads to the low number of taxa. High rainfall was seen in September and October, during these months taxa richness becomes higher compared to non-rainy months.

Table 1. Ephemeroptera species recorded in the Chinnasuruli stream during different months

Family/ Species	Number collected						
	Aug	Sep	Oct	Nov	Dec	Jan	Total
Baetidae							
Baetis conservatus Müller-Liebenau & Hubbard, 1985	5	11	9	8	7	6	46
Tenuibaetis frequentus Muller-Liebenau & Hubbard, 1985	2	9	10	7	8	5	41
Acentrella vera Müller-Liebenau, 1982	0	7	6	9	5	4	31
Heptageniidae							
Afronurus kumbakkaraiensis Venkataraman & Sivaramakrishnan, 1990	1	1	2	3	1	0	8
Epeorus petersi Sivaruban, Venkataraman & Sivaramakrishnan, 2013	2	8	11	9	7	4	41
Thalerosphyrus flowersi Venkataraman & Sivamarakrishnan, 1987	1	2	6	4	4	2	19
Leptophlebiidae							
Choroterpes alagarensis Dinakaran, Balachandran & Anbalagan, 2009	11	14	18	16	12	7	78
Choroterpes nambiyarensis Selvakumar, Arunachalam & Sivaramakrishnan, 2013	4	12	12	11	8	4	51
Choroterpes sp.	2	3	5	7	3	1	21
Isca sp.	0	3	4	2	2	1	12
Teloganodidae							
Derlethina sp.	0	0	2	5	2	1	10
Dudgeodes palnius Selvakumar, Sivaramakrishnan & Jacobus, 2014	9	6	8	9	4	2	38
Dudgeodes sartorii Srinivasan, Sivaruban, Barathy & Isack 2021	5	7	9	11	6	1	39
Teloganodes kodai Sartori, 2008	7	9	6	7	6	4	39
Teloganodes sp.	2	1	3	6	5	2	19
Caenidae							
Caenis sp.	7	2	0	0	1	6	16
Clypeocaenis bisetosa Soldán, 1978	4	3	2	1	1	3	14
Total	62	98	113	115	82	53	523

Table 2. Diversity indices of Chinnasuruli stream during different months

Indices	Aug	Sep	Oct	Nov	Dec	Jan
Taxa_S	14	16	16	16	17	16
Individuals	62	98	113	115	82	53
Simpson index	0.8959	0.9107	0.915	0.9211	0.9191	0.9163
Shannon index	2.415	2.544	2.597	2.632	2.637	2.597

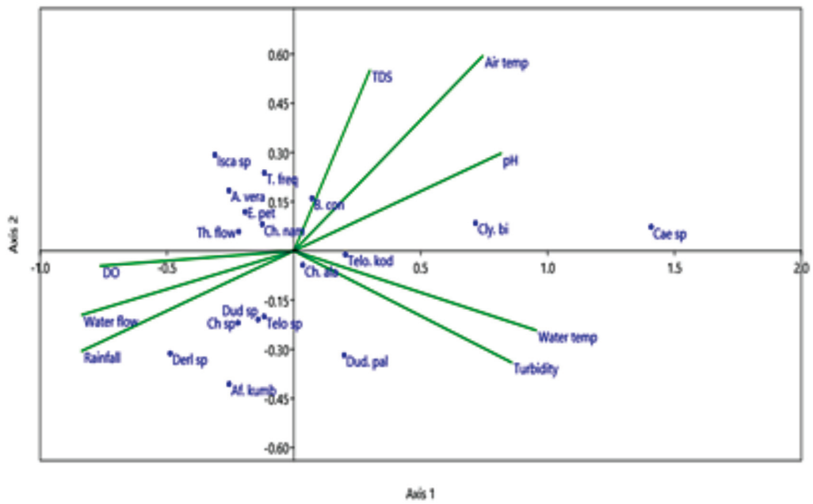

Fig. 1 Canonical Correlation Analysis (CCA) of Ephemeroptera in correlation with ecological attributes in Chinnasuruli stream.

(B. cons- *Baetis conservatus*, T. freq- *Tenuibaetis frequentus*, A. vera- *Acentrella vera,* Af. kum -*Afronurus kumbakkaraiensis*, E. pet- *Epeorus petersi,* Th flo- *Thalerosphyrus flowersi,* Ch. ala- *Choroterpes alagarensis*, Ch. nam- *Choroterpes nambiyarensis*, Ch sp- *Choroterpes* sp., Isca sp- *Isca* sp., Telo. kod- *Teloganodes kodai,* Telo sp- *Teloganodes* sp., Dud. Pal- *Dudgeodes palnius*, Dud sp- *Dudgeodes sartorii*, Derl sp- *Derlethina* sp., Cae sp- *Caenis* sp., Cly. bi- *Clypeocaenis bisetosa*)

Table 3. Physico-chemical parameters of Chinnasuruli stream during different months

Indices	Aug	Sep	Oct	Nov	Dec	Jan
Water temperature (°C)	22.4	21.1	20.7	20.5	20.5	21.4
Air temperature (°C)	27.5	27.6	27.2	27.0	27.2	27.8
Water flow (m\s)	0.50	0.52	0.74	0.72	0.68	0.54
Water pH	7.4	7.3	7.3	7.3	7.3	7.5
Dissolved oxygen (mg/l)	7.2	7.3	7.9	8.1	8.2	7.6
Total dissolved solids (ppt)	0.15	0.14	0.15	0.15	0.18	0.21
Turbidity (NTU)	0.30	0.21	0.16	0.15	0.15	0.19
Mean monthly rainfall (mm)	21.11	78.42	221.71	287.62	145.72	30.21

Canonical correspondence analysis (CCA) determines the correlation between EPT communities and environmental variables (TerBraak and Smilauer, 2002). CCA biplot reveals that taxa such as *Caenis* sp., *Baetis conservatus* Müller-Liebenau & Hubbard, 1985 and *Clypeocaenis bisetosa* Soldán, 1978 prefer high pH, air temperature, and total dissolved solids for their survival and they were inversely proportional to high rainfall, DO, and water flow (Fig. 1). High DO, rainfall, and water flow enhance the taxa like *Afronurus kumbakkaraiensis* Venkataraman & Sivaramakrishnan, 1990, *Choroterpes* sp., *Derlethina* sp. and *Dudgeodes* sp. and they were sensitive to attributes like pH, air temperature, and TDS. Taxa include *Tenuibaetis frequentus* Muller-Liebenau & Hubbard, 1985, *Acentrella vera* Müller-Liebenau, 1982, *Epeorus petersi* Sivaruban, Venkataraman & Sivaramakrishnan, 2013, *Thalerosphyrus flowersi* Venkataraman &

Table 4. Correlations of environmental gradients with the axes of CCA

Variables	Axis 1	Axis 2
Water temperature	0.954458	-0.24214*
Air temperature	0.743874	0.593868
Water flow	-0.83485*	-0.19507*
pH	0.81631	0.296973
DO	-0.7624*	-0.04579*
TDS	0.299409	0.549704
Turbidity	0.857756	-0.34013*
Rainfall	-0.83505*	-0.30507*

*indicates significant differences

Sivamarakrishnan, 1987, *C. nambiyarensis* Selvakumar, Arunachalam & Sivaramakrishnan, 2013 and *Isca* sp. gets affected by high levels of turbidity and water temperature whereas *C. alagarensis*, *Teloganodes kodai* Sartori, 2008 and *Dudgeodes palnius* Selvakumar, Sivaramakrishnan & Jacobus, 2014 gets nourished by high levels of turbidity and water temperature and *C. alagarensis* becomes the most diverse and tolerant taxa in the Chinnasuruli stream of Western Ghats. Rainfall, water flow, turbidity, and air temperature are vital in governing the diversity and distribution of mayfly larvae in Chinnasuruli stream (Table 4). Beyene *et al.* (2008) found that rainfall turns into a major element in governing the mayfly diversity and our results also show a closer resemblance to their findings. This study revealed that *C. alagarensis* is the most dominant taxon in the Chinnasuruli stream of the Western Ghats and environmental characteristics such as precipitation, water flow, turbidity, and air temperature are the main components in managing mayfly distribution.

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Species composition and host preference of fleas (Insecta: Siphonaptera) on rodent and domestic animals in Tamil Nadu, India

V. Rajamannar, R. Govindarajan, R. Krishnamoorthi[#], Ashwani Kumar[#] and P. Philip Samuel[*]

ICMR - Vector Control Research Centre Field Station, Division of Vector-Borne and Zoonotic Diseases, Department of Health Research, Madurai 625002, Tamil Nadu, India;
[#]ICMR - Vector Control Research Centre, Indra Nagar Puducherry, India.
Email:philipsamuelpaulraj@gmail.com

ABSTRACT: The species composition and host preference of medically important fleas monitored in urban, semi-urban, and rural revealed 412(65%) and 222(35%) fleas. From urban and rural habitats 90 and 345 fleas were collected respectively. There was a significant difference between urban and rural habitats in flea abundance. From rodents and domestic animals 209 (33%) *Xenopsylla cheopis*, 203 (32%) *X. astia* and 222 (35%) *Ctenocephalides felis* fleas were recorded. Fleas were predominantly found on *Rattus rattus* 45(83.3%) and *Canis familiaris* 31(83.8%). Among the habitats, there was no significant difference in rodent flea positivity and dog/cat flea positivity.

KEYWORDS: Habitats, abundance, positivity, flea infestation rate, flea index

INTRODUCTION

Pulicidae (with genus *Pulex, Ctenocephalides, Pilopsyllus* and *Archaeopsyllus*) and Ceratophyllidae (with genus *Ceratophyllus* and *Nosopsyllus*) are important fleas distributed worldwide (Durden and Hinkle, 2009). About 2652 species belonging to 18 families, 27 subfamilies, and 238 genera have been described (Hastriter and Bossard, 2018). Fleas are important vectors to plague and murine typhus diseases in many parts of the world (Durden and Hinkle, 2009). Fleas prefer the blood of warm-blood mammals and birds. Both sexes of fleas are obligate hematophagous prefers blood from the host animals, act as an ectoparasitic vector. A total of 46 species and 5 subspecies belonging to 24 genera under eight families were described in India (Chandra *et al.*, 2018). In the Indian Himalayan region, 38 species of fleas belonging to 22 genera of seven families were described which were sylvatic, collected from the wild rodents and animals (Chandra *et al.*, 2018). Fleas associated with human dwellings act as vectors and transmit flea-borne diseases to humans. This study was planned to find out the species composition of the fleas and vertebrate host preference of medically important fleas in the Madurai district south Tamil Nadu.

* Author for correspondence

MATERIALS AND METHODS

Study sites: Madurai district is located in south Tamil Nadu of India, lies between 9°33'30''N to 10°18'50'' N latitude, 77°29'10' 'E to 78°28'45'' E longitude and has an area extent of 3710 sq. km (https://madurai.nic.in/district-profile/). Nine study sites were selected, grouped into urban, semi-urban, and rural habitats with three sites each as B.B.Kulam, Tirumangalam, Usilampatti (all three in urban habitats), Peraiyur, Keelaiyur, Sholavandan (all three in semi-urban habitats), Vadapalanji, Katchaikatti, and Chatrapatti (all three in rural habitats).

Collection of fleas

From rodents: At every site, before the dusk hours (5-6 pm), Sherman traps (width 7.5 cm, length 18.5 cm, and depth 9 cm) (Sadanandane *et al.,* 2016; Philip Samuel *et al*., 2020, 2021a,c) were kept in and around residential areas in indoor and outdoor households and withdrawn after dawn (6-7 am) in the next day. All the rodents were attracted by fried eatables smeared with coconut oil kept within the Sherman traps and captured. The design of the Sherman trap was made to capture only a single rodent at a time and, after trapping a single rodent, the door of the trap will close automatically (Philip Samuel *et al*., 2020; 2021a). Captured pest rodents and shrews were identified based on external morphology (Shakunthala and Tripathi, 2005; Martin, 2011).

To collect various small rodents, 1080 Sherman traps were placed in the study sites during the study period from July 2017 to June 2018, as a total of 360 Sherman traps were placed in each urban, semi-urban and, rural habitat (i.e., 120 traps were placed/site/year in the 9 study sites). For every month, 9 visits were made with three sites each from the urban, semi-urban, and rural habitats selected, for the collection of rodent fleas. All the trapped rodents were placed in separate cloth bags and brought to the laboratory. Captured rodents were anesthetized for the collection of fleas (Kreeger and Arenemo, 2012; Philip Samuel *et al.,* 2020, 2021 a,b,c).

Fleas from domestic and companion animals: From each habitat, 100 households were selected randomly. The study incorporated different households with domestic and companion animals like cats, dogs, goats, and fowls were sampled for flea collection. Fleas were collected manually from the body of the host animals by combing the hair using an aspirator or fine brush and kept separately in sample vials containing 70 per cent alcohol for identification provided with thelocation and date of fleas collected. Fleas were identified using available identification keys (Shariff, 1930; Iyenger 1973). Fleas were mounted with Hoyer's medium (Taylor *et al.,* 2007; Ashwini *et al.,* 2017a) and all collected specimens were deposited in the Mosquito and Ectoparasite Museum, Entomology laboratory of ICMR-Vector Control Research Centre Field station, Madurai, Tamil Nadu, India. This study was approved by the Institutional Animal Ethical Committee (IAEC) of ICMR-Vector Control Research Centre, Puducherry.

Data analysis: Data were analyzed by using computer software IBM SPSS Statistics Ver.25, applied for statistical calculations like chi-square analysis to test significant differences in species distribution and host preference in the study locations. Estimation of rodent flea and domestic animal flea infestations were calculated based on the calculated index (Shelly *et al.,* 2013). Flea infestation rate and flea index were calculated as -

$$\text{Flea infestation rate (FIR)} = \frac{\text{Total number of animals with flea}}{\text{The total number of animals examined}} \times 100$$

$$\text{Flea index (FI)} = \frac{\text{Total number of flea collected}}{\text{The total number of animals examined}} \times 100$$

For GPS-based spot mapping, study site distance and location measure, Epi Map of Epi Info Ver. 7.2.2.6 of CDC, Atlanta, USA powered by ESRI was used. Indian states and district-level maps were downloaded from the website of www.d-map.com.

RESULTS AND DISCUSSION

From the trapped 151 rodents, 35(23%), 51(34%), and 65(43%) rodents were collected from urban, semi-urban, and rural sites respectively. Only 54 rodents were positive for fleas (36%). *Rattus rattus* (Linnaeus, 1758) was trapped more in urban (69%), semi-urban(59%), and rural (63%) sites respectively. *Tatera indica* Hardwicke, 1807 trapped in rural areas only. *Bandicota bengalensis* (Gray and Hardwicke, 1833) collected at rural sites and *R. norvigecus* (Berkenhout, 1769) collected at semi-urban areas showed high flea infestation rate and high flea index there was no significant difference in rodent flea positivity at three different habitats ($\chi^2 = 3.9008$, df = 2, P>0.05). In all the habitats, a high number of rodent flea was collected from *R. rattus.* A total of 412 (65%) of *Xenopsylla cheopis* (Rothschild, 1903) and *X.astia* Rothschild, 1911) were collected from all study sites. From all the study sites, a total of 412 fleas were collected from positive rodents trapped. Fleas were collected as 60 (14.56%) from urban, 139 (33.73%) from semi-urban, and 213 (51.70%) from rural areas (Fig. 1). Flea infestation rate was 6.00, 8.69, and 7.61, and the flea index was 1.71, 2.73, and 3.28 for urban, semi-urban, and rural sites respectively (Table 1 & 2). The rural site was showing a high flea index (3.28).

Among 735 domestic and companion animals examined, only 37 (5%) animals were found positive for fleas; 31 (83.8%) dogs *Canis familiaris* Linneaus, 1758 and 6 (16.2%) cats (*Felis catus* Linnaeus, 1758) were positive for fleas. A total of 222 fleas were collected from these 37 animals and all the collected fleas were *Ctenocephalides felis* (Bouché, 1835). From urban, semi-urban and rural habitats *C. felis* collection was 13.51, 27.03 and 60.05 percent respectively. Rural dogs showed high flea index of 1.88 (Table 2) and there was no significant difference in fleas collection from dogs and cats at different sites ($\chi^2 = 0.4884$, df = 2, P>0.05) Fowls and goats were tested negative for all the fleas. In all the study sites, 20 *X. cheopis* (33%), 203 *X. astia* (32%) and 222 *C. felis* fleas (35%) were collected and *X. astia* was collected more in rural sites. Only 90 fleas were in the urban site. However, 345 fleas (54%) were collected in rural, which was 3.83 times higher than the urban collection and showed a significant difference between urban and rural fleas

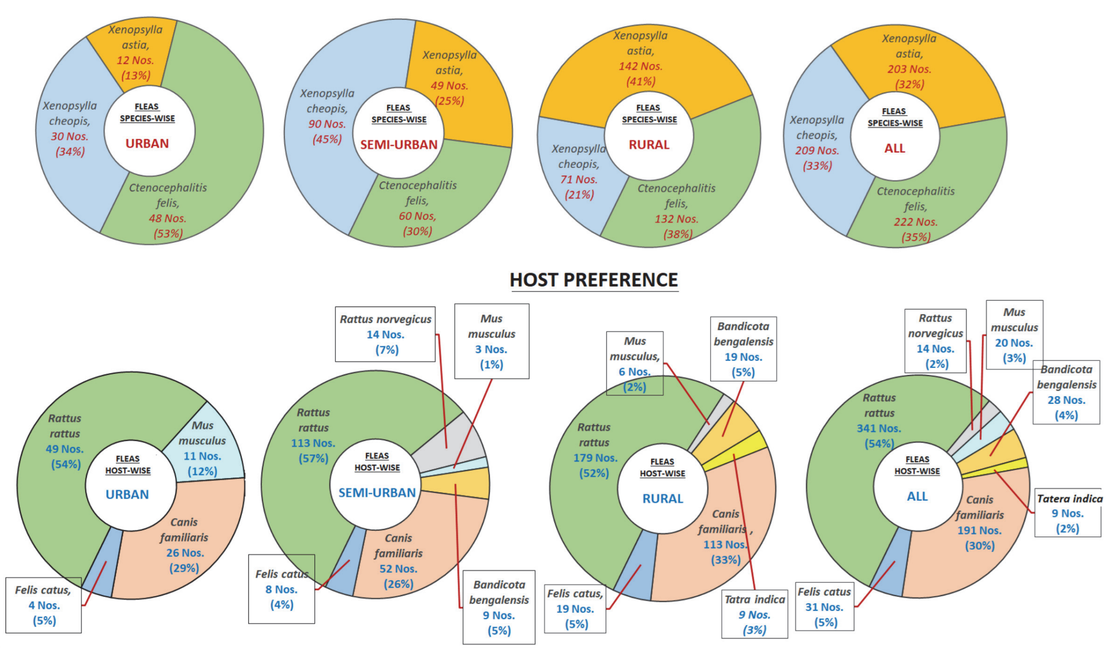

Fig. 1 Species composition and host preference of fleas collected in south India

Table 1. Rodents trapped and flea positivity in south Tamil Nadu (July 2017– June 2018)

Areas	Rodents / shrews	Trap positivity rate	Flea infestation rate	Flea index
Urban	*Rattus rattus*	6.67	6.13	2.04
	Rattus norvigecus	0.28	0.00	0.00
	Mus musculus	1.11	5.50	2.75
	Suncus murinus	1.39	0.00	0.00
	Bandicota bengalensis	0.28	0.00	0.00
	Total	9.72	6.00	1.71
Semi Urban	*Rattus rattus*	8.33	8.69	3.77
	Rattus norvigecus	0.28	14.00	14.00
	Mus musculus	1.39	3.00	0.60
	Suncus murinus	3.61	0.00	0.00
	Bandicota bengalensis	0.56	9.00	4.50
	Total	14.17	8.69	2.73
Rural	*Rattus rattus*	11.39	7.46	4.37
	Rattus norvigecus	0.28	0.00	0.00
	Mus musculus	0.83	6.00	2.00
	Suncus murinus	3.89	0.00	0.00
	Bandicota bengalensis	0.83	9.50	6.33
	Tatera indica	0.83	9.00	3.00
	Total	18.06	7.61	3.28

collected ($t = 2.229$, $df = 33$, $p < 0.05$). But, there was no significant differences between semi-urban and rural fleas collected ($t = 1.127$, $df = 542$, $p > 0.05$) and semi-urban and urban fleas collected ($t = 1.593$, $df = 287$, $p > 0.05$).

Female fleas were more than males, in all sites. But, there was no significant difference between a sex-wise collection of fleas ($t = 1.535$, $df = 632$, $p < 0.05$). In all habitats, 248 male (39%) and 386 female fleas (61%) were collected which showed there was no significant difference between male and female fleas collection ($t = 0.477$, $df = 632$, $p < 0.05$). *Xenopsyalla astia, X. cheopis,* and *C. felis* were collected more or less equally from all study sites (Table 3a). However rural habitat showed high flea infestation as high as 345 fleas (54%) (Table 3b). Flea infestation by *X. astia, cheopis* and *C. felis* was 16.6, 14.8, and 11.5 percent respectively (Table 4).

Plague outbreaks were observed during 1994 in Maharashtra, Gujarat, Uttar Pradesh, and Delhi by commensal rodents like *R. rattus, R. norvegicus, M. musculus, T. indica, S. murinus, B. bengalensis,* and *B. indica* and only 41.64 of rodents were found positive for plague vectors, *X. astia,* and *X. cheopis*. In the Chittoor district, Andhra Pradesh 62 per cent *X. astia* and 38 per cent *X. cheopis* were reported from rural, semi-urban, and urban habitats during the plague outbreak (Shelly *et al*., 2013). *X. cheopis* fleas also act as a vector for Murine typhus (Azad, 1990). The fleas, *X. cheopis*, and *X. astia* were reported from the commensal rodents (Kumar *et.al*, 1997). *X. cheopis* and *X. astia* are now common in Indian rodents. In India, many studies were also carried on the diversity and bionomics of rodent fleas, their host preference and plague disease (Shelly *et al*., 2013). Plague and Murine typhus is still the major vector-borne disease transmitted by rodent fleas

Table 2. Flea infestation (%) and index on domestic animals in south Tamil Nadu (July 2017 –June 2018)

Areas	Host	Infestation	Index
Urban	Dog	11.90	0.62
	Goat	0.00	0.00
	Cat	11.11	0.44
	Fowls	0.00	0.00
	Total	3.70	0.19
Semi-Urban	Dog	15.69	1.02
	Goat	0.00	0.00
	Cat	33.33	1.33
	Fowls	0.00	0.00
	Total	4.72	0.28
Rural	Dog	30.00	1.88
	Goat	0.00	0.00
	Cat	27.27	1.73
	Fowls	0.00	0.00
	Total	5.82	0.37

Table 3a. Sex-wise flea species collected (number/ per cent) in south Tamil Nadu

Species	Male	Female
Xenopsylla astia	85(42%)	118(58%)
Xenopsylla cheopis	86(42%)	123(58%)
Ctenophalides felis	77(35%)	145(65%)
Total	248(39%)	386(61%)

Table 3b. Distribution of fleas (number and per cent wise) at the different habitats in south Tamil Nadu

Habitat	Male	Female
Urban	33(37%)	57(63%)
Semi-urban	78(39%)	121(61%)
Rural	137(40%)	208(60%)
Total	248(39%)	386(61%)

Table 4. Prevalence of flea species and host preference in south India

Host	Flea species					
	Xenopsylla astia		*X. cheopis*		*Ctenophalides felis*	
	No. host positive (%)	No. fleas	No. host positive (%)	No. fleas	No. host positive (%)	No. fleas
Rattus rattus	27(14.5)	186	18(11.6)	155	0	0
Rattus norvigecus	0	0	1(7.1)	14	0	0
Mus musculus	1(3.0)	3	3(17.6)	17	0	0
Bandicota bengalensis	1 (5.0)	5	2(8.7)	23	0	0
Tatera indica	1(9.0)	9	0	0	0	0
Canis familiaris	0	0	0	0	31 (16.0)	191
Felis catus	0	0	0	0	6 (19.3)	31
Total	30 (14.8)	203	24 (11.5)	209	37(16.6)	222

Note: (%) indicates flea infestation percentage of hosts

and in India. *C. canis,* and *C. felis* were reported from dogs at Shimoga district, Karnataka (Krishna Murthy *et al.,* 2017). The fleas, *C. canis,* and *C. felis* are common in dogs and cats (Durden *et al.*, 2005).

These flea species are occasionally found on other rodents and domesticated animals (Biswas, 2018). *X. astia* is restricted in its distribution mainly in India and the oriental region, distributed widely at peri-domestic areas and wild situations (Biswas, 2018;

Philip Samuel *et al*., 2020, 2021a). In the Madurai district, urban, semi-urban and rural habitats were rich with domestic and peri-domestic rodents favors to the distribution of *X. astia* and *X. cheopis* vector fleas. *X. astia* (49%) and *X. cheopis* (51%) were collected from all sites 412(65%) of *Xenopsyalla species* fleas and 222(35%) of *C. felis* fleas from dogs and cats were collected in all the study sites, indicating more rodent vector fleas distributed showing risk for rodent based vector-borne diseases. In Madurai study sites, *C. felis* in the dogs and *X. astia* in the rodents were collected abundantly which may cause flea-borne diseases at any time. *C. felis* is predominant in dogs and cats worldwide and in India, the prevalence of *C. felis* was also confirmed in the distribution (Iyengar, 1973). *Ctenocephalides* fleas are still the source of rickettsial diseases worldwide, particularly for *Rickettsia felis* (Hii *et al*., 2015). No published evidence is available distribution of *R. felis* in India till now.

Ctenocephalides orientis is the widely distributed on ruminants and less commonly on dogs and cats in India (Taylor *et al*., 2007). Likewise, many *Ctenocephalides* spp. were reported from the goats (Kaal *et al*., 2006, Obasaju and Otesile, 1980). Some fowls in Indian poultry farms were also severely affected by the bite of fleas, identified as *C. felis* (Joseph *et al*., 1987). *Ctenocephalide*s serve as vectors for *R. felis* which causing some rickettsial zoonotic diseases. *Rickettsia* sp. genotype RF 2125 is one of the dominant rickettsiae carried by Dog fleas *C. felis orientis* was confirmed in India for rickettsial zoonotic diseases (Hii *et al*., 2015). In Tamil Nadu, severe goat infestations by fleas were recorded (Soundararajan *et al*., 2018). Animal workers and even veterinarians were also affected by the fleas and flea bites with allergic dermatitis symptoms that were common, transmitted through the bite of goat fleas (Soundararajan *et al*., 2018).

Murine (endemic) typhus are flea-borne infectious disease caused by *R. typhi* transmitted by the rat flea, *X. cheopis* (Azad, 1990). Recent serological and molecular evidence confirmed the presence of Murine typhus pathogen, *R. typhi* in the fleas responding to the transmission of the disease to humans (Rakotonanahary *et al*., 2017) in India. The presence of Murine typhus pathogen was also observed as a cross-sectional survey, conducted in Vellore, Thiruvannamalai, and Salem districts, all in Tamil Nadu, India, at three different geographic areas such as urban, rural plains, and rural hill areas (Devamani *et al*., 2020).

Based on earlier records of fleas abundance and prevalence, outbreaks of plague coccured in India at different habitats. This study was carried out to observe the species composition and host preference of medically important fleas located in the Madurai district and showed flea species like *X. astia, X. cheopis,* and *C. felis* which were found associated with the transmission of the diseases like Plague, Murine typhus, and Flea-associated dermatitis.

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Comparative studies on *Culex bitaeniorhynchus* Giles (1901) and its *tenax* variant (Diptera: Culicidae) in Chandigarh, India

Rani Ranjana[1], Kaur Sandeep[2], Kaur Sukhbir[2] and Kaur Sagandeep[1*]

[1]P.G. Department of Zoology, DAV College, Sector-10, Chandigarh, India; [2]Department of Zoology, Panjab University, Sector-14, Chandigarh, India. Email: contact.93ranjana@gmail.com[1], sainiruby17@gmail.com[2], sukhbir@pu.ac.in[2], mailtoshagan@yahoo.co.in[1]*

ABSTRACT: During present investigations the detailed morphotaxonomic studies of *Culex bitaeniorhychus* revealed that it exists in two forms *i.e typical* and *tenax* in and around Chandigarh. Various intraspecific variations in the morphology and male genitalia were observed in both these forms. The main aim of this study is to distinguish these two forms of *Cx. bitaeniorhynchus* and their separation from other closely related species. The intraspecific variants of these forms have been studied further with respect to phallosome regions of the male genitalia. For assessing the significant differences among their phenotypic characteristics one–way ANOVA was done along with pair-wise comparisons of samples means. Some of the earlier workers have considered *tenax* form, a synonym of *Cx. infula* which is another closely related species of *Cx. bitaeniorhynchus.* But, on the basis of remarkable differences observed between *tenax* and *infula*, it is suggested that these two are separate taxons and should not be synonymised.

KEY WORDS: *Culex bitaeniorhynchus*, *Cx. infula, typical*, intraspecific variation

INTRODUCTION

Culex (Oculeomyia) bitaeniorhynchus Giles (1901) complex has six closely related species viz; *Culex bitaeniorhynchus*, *Cx. infula*, *Cx. longicornis*, *Cx. luzonensis*, *Cx. pseudosinensis* and *Cx. selangorensis*. Several species of this subgroup are widely distributed from Southeast to Southwest Asian countries like Africa, Egypt, Japan, Korea and Eastern Palearctic regions (Tanaka, 1979; Sirivanakarn, 1976; Harbach 1988; Reuben 1994; Tanaka, 2004), and in Indian subcontinents, particularly India, Bangladesh, Nepal, and Pakistan. *Culex bitaeniorhynchus* is well known as a rural species which breed in marshes, puddles, rice fields and the habitats rich in water containing Spirogyra, a filamentous green-algae (Harbach, 1988; Sirivanakarn, 1976). It has a major role in spreading various arboviral diseases like Japanese encephalitis, filariasis in tropical and subtropical areas of Asia (Iyengar 1938; Carter 1948; Benerjee et al. 1975, 1978; Reuben et al. 1994). *Cx. bitaeniorhynchus* is a highly plastic species which shows intraspecific variations in both of its morphology and male genitalia. These variations have been observed with respect to erect scales on center of vertex of head, thorax, legs, wings, apical bands of abdominal terga III–IV and inner

* Author for correspondence

and outer division of phallosome region of male genitalia. Previous researchers have reported considerable variations in *Cx. bitaeniorhynchus* and proposed these variations as subspecies, varieties or forms of *Cx. bitaeniorhynchus* (Edwards, 1922; Barraud, 1934; Bram, 1967; Sirivanakarn, 1973). This species group also includes many binomial names and hence due to the complexity of various synonyms (Sirivanakarn, 1976), it is difficult to distinguish or classify the species accurately.

During present communications, morphotaxonomic studies were made on *Cx. bitaeniorhynchus* from Chandigarh and its surrounding areas. In addition to morphological variants the allied form '*tenax*' was also found in the area. *Cx. bitaeniorhynchus* (including form '*tenax*') was found widely distributed in most areas of Chandigarh. In India, no one has made any attempt to study '*typical*' and its allied '*tenax*' form of *Cx. bitaeniorhynchus* along with intraspecific variations. It was Barraud (1934) who for the first time replaced three forms *i.e* '*ambiguus*', '*tenax*' and one unnamed *var*. into varieties from Indian region. After almost decades, the taxonomic studies have not been made in India. Thus, in current analysis, various phenotypic attributes have been re-examined in detail in 154 samples from study areas, under three years of surveillance to explore its variability. The morphometric analysis of distinguishing characters on the phallosome of male genitalia of *Cx. bitaeniorhychus* and its '*tenax*' form were also done to know the exact taxonomic status.

MATERIALS AND METHODS

Study area: The Chandigarh, which is located near foothills of the Shivalik range of Himalayas in Northwest India (30.74º N, 76.79º E) has variety of favourable habitats for mosquito breeding like thick vegetation which cover around 8.77 per cent of total geographical areas, 3245.30 hectares forest area, green belts of gardens, lakes full of flora and fauna (also attracts various species of migratory birds from parts of Siberia and Japan in the early winter season), paddy fields and slum areas on its outskirts. There are different seasons like summer (March–May), pre-monsoon (June–July), monsoon (August–September), post-monsoon (October–November) and winter (December–Mid March). The surveys were conducted during the transitional period of the season from pre-monsoon to post-monsoon seasons.

Mosquito collections, Identification and analysis: Daily visits were made from different categorized habitats *viz*; Developed areas, Garden belts, Villages and Slums of Chandigarh (Fig. 1). Adult specimens were collected from different resting sites near temporary or permanent urban pools, grasses, gardens, slum areas, villages having cattle sheds, and in and around pig enclosures, with the help of mouth aspirators and hand net traps under three years of entomological surveillance from June 2017– November 2019. The adults were collected during dawn and dusk from indoor and outdoor resting places. Field–caught samples were held in the laboratory to record the variability and distribution pattern. Adult specimens were preserved in insect collection boxes after pinning and identified with the help of identification keys (Barraud, 1934; Sirivanakarn, 1973, 1976). Siverly & Shroyer (1974) methodology was adopted to prepare slides for male genitalia. The terminology used for naming different parts of male genitalia was revealed by Sirivanakarn and Reuben. After careful identification, the adults were photographed under Stereo Zoom Trinocular Microscope attached with digital camera. Slides of male genitalia were studied and photographed under research microscope. The morphometric measurements of phenotypic attributes of phallosome were studied using compound microscope calibrated with stage micrometer and digital camera. Further, for statistical analysis, the mean (M) and Standard deviations (SD) of the various parts of phallosome was computed by parametric tests using one–way ANOVA.

RESULTS

During present work, 154 adults of *Cx. bitaeniorhynchus* were collected from four majorly categorized habitats *viz*; developed urban areas, villages, garden belts and slums of Chandigarh and its surrounding areas and identified. It has been

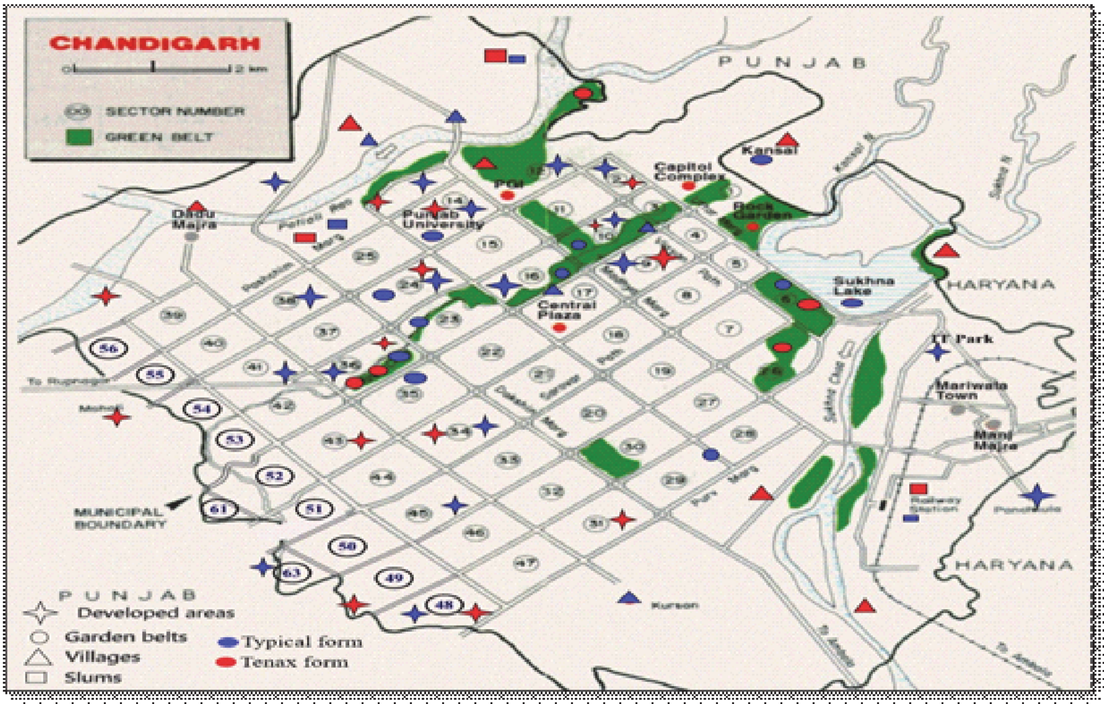


Fig. 1 Map of Chandigarh showing various ecological habitats surveyed during Jun 2017–Nov 2019.

Developed area		Garden belts	Villages		Slums
1.Sector4	16.Baltana	1.Bougainvellia Garden	1.Sector4	16.Baltana	1.Khuda Lahora
2.Sector 8	17.Sector 31	2.Leisure Valley	2.Sector 8	17.Sector 31	2.Mauli Jagran
3.Sector14	18.Sector 40	3.Rose Garden	3.Sector14	18.Sector 40	3.Bapu Dham Colony I
4.Sector 15	19.Bal Bhawan	4.Fragnance Garden	4.Sector 15	19.Bal Bhawan	4.Palsora
5.Sector 38	20.Mohali	5.Terrace Garden	5.Sector 38	20.Mohali	5.Burail
6.Sector 39	21.Nada sahib	6.Topiary Garden	6.Sector 39	21.Nada sahib	6.Dadu Majra
7.Sector 46	22.Panchkula	7.Pointsettian&Ixora	7.Sector 46	22.Panchkula	7.Sector 25
8.Sector 48	23.Zirakpur	8.Botanical Garden (Sec-14)	8.Sector 48	23.Zirakpur	8.Madanpur
9.Sector 2	24.Manimajra	9. Hibiscus Garden	9.Sector 2	24.Manimajra	9.Raipur khurd
10.Sector 9	25Sukhna Lake	10.Dahlia Garden	10.Sector 9	25.Sukhna Lake	10.Makkanmajra
11.Sector 16	26.New Lake	11.Garden of Silence	11.Sector 16	26.New Lake	11.Railway Colony
12.Sector 24	(&other areas)	12. Butterfly Garden	12.Sector 24	(&other areas)	12. BapuDham Colony II
13.Sector 34		13.Valley of Animal	13.Sector 34		
14.Sector 36		14. Garden of Springs	14.Sector 36		
15.Sector 45		15. Garden of Palms	15.Sector 45		
		16. Japanese Garden			
		(& more)			

Abbreviations used:

SAL – Sub Apical Lobe
ASP – Apical Sternal Speculate Process
BSP – Basal Sternal Processes
ASA – Apical Sternal Angle
LBP – Lateral Basal Process
ASSP – Apical Sternal Spiculate Portion/ Inner Division
GC-–Gonocoxites
GS–Gonostylus

PPR –Paraproct
FP –Folliform Processes
ATA – Apical Tergal Angle
OD – Outer Division
AM – Apical Margin
TE – Terga

P – Proboscis
MF – Mid Femur
FF – Fore Femur
W – Wing
ES– Erect Scale

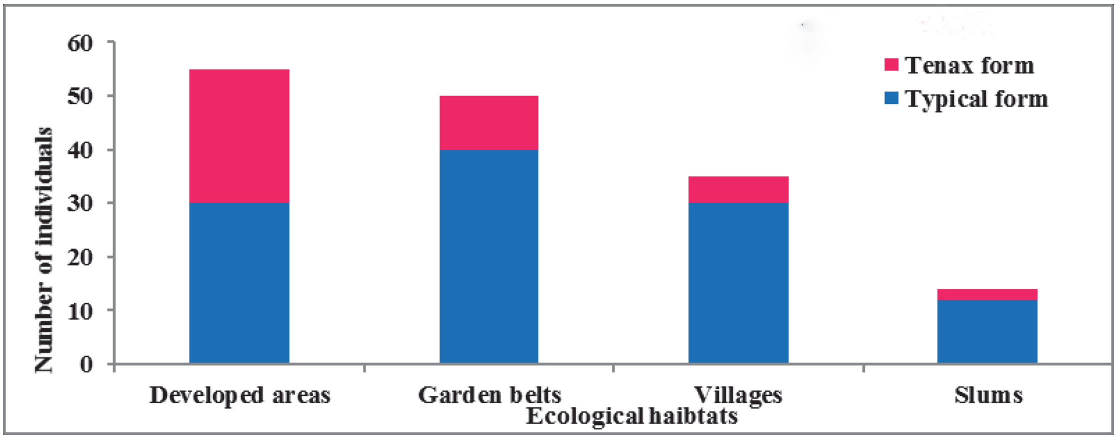


Fig. 2 Ecological distribution of *Culex bitaeniorhynchus* typical and *tenax* from Chandigarh (June 2017-Nov 2019)

observed that *Cx. bitaeniorhychus* exists as both '*typical'* as well as '*tenax'* form, which comprised 72.7 and 27 per cent respectively. Out of these four ecologically divided habitats, both *typical* and *tenax* has shown maximum abundance in developed areas (47.4%), followed by garden belts (31.8%), villages (16.2%) and slums (4.54%) during three years i.e June 2017–November 2019 (Fig. 2). These adult populations started appearing in June, showed an upward trend in July and then reached to peak in August. Thereafter, population density declined during September and October and greatly reduced in November.

The taxonomic investigations of both *typical* and *tenax* form of *Culex bitaeniorhynchus* along with their intraspecific variations have been done in detail in the present communication. It has been observed that these two forms differ with respect to the erect scales on center of vertex of head, colour of scales on scutum of thorax, speckling of scales on the legs as well as on the wings, apical and basal bands on abdominal terga and the phallosome attributes of male genitalia. The phenotypic differences in the morphology of respective forms '*typical'* and '*tenax'* along with their intraspecific variants has been elucidated (Table 1, 1a).

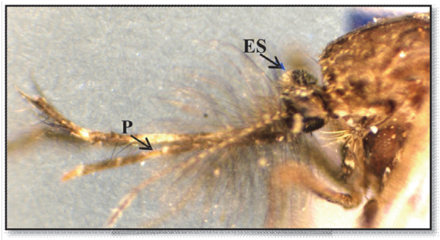
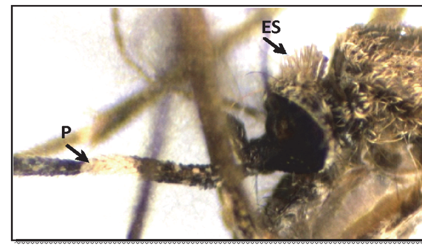
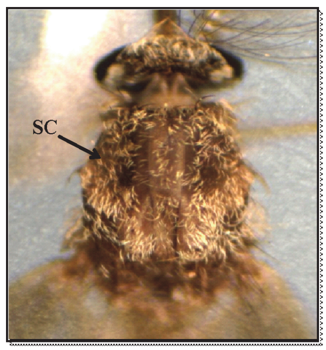
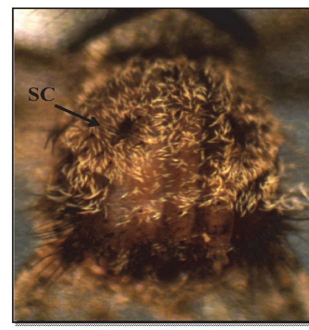
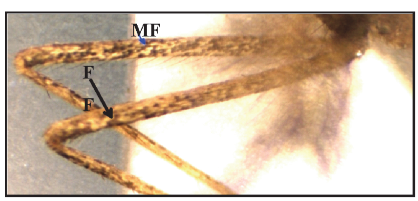
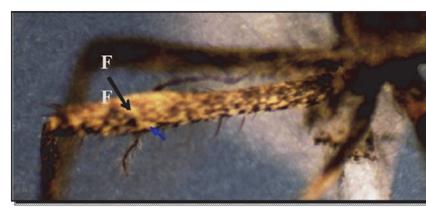
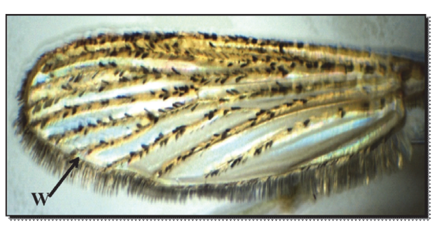
Male Genitalia (♂)

In *Cx. bitaeniorhynchus,* the gonocoxites (**GC**) are slender or conical in shape and gonostylus (**GS**) are elongate with apex sometimes swollen in shape and has long spiniform setae and hair majorly on lateral sides. Sub Apical Lobe (**SAL**) of gonostylus is small with a basal rod like structure. Apical region of Paraproct (**PPR**) is dark and covered with setae. In phallosome, apical speculate portion (**ASP**) of inner division to apex is typically beaklike sharp, vary in length, along with smoothly curved apical margin. Apical tergal angle blunt; basal sternal process of proctiger slender and vary in length, while the apical sternal angle strongly produced sterned into sharp beak; inner tergal surface with distinct lobe bearing 2–3 budlike processes called folliform Processes (**FP**). Outer division (OD) is in shape of broad acuminate leaf with variable structure; lateral basal processes (LBP) is mostly knoblike (Fig. 4A, Fig. 4B).

Lateral Plate of Phallosome: The lateral plate of phallosome of '*typical'* and '*tenax'* along with their intraspecific variations is described below.

'*Typical'* (Fig. 5A): Apical Sternal Angle **(ASA)** of apical sternal speculate portion of inner division strongly produced into typically sharp sterned beak, 0.96mm in length, covering with smoothly curved apical margin. Apical Tergal Angle **(ATA)** is satiny curved; Basal Sternal Lobe (**BSL**) of proctiger slender, vary in length, while the inner tergal surface with distinct lobe bearing 2–3 budlike fused Folliform processes (**FP**). Outer division (**OD**) is in shape of broad acuminate leaf with average 0.591mm

Table 1. Comparative phenotypic details of morphotaxonomy of *'typical'* and *'tenax'* form of *Culex bitaeniorhynchus*

	'Typical' form	*'Tenax' form*
Head		
Thorax		
Legs		
Wings		

horizontal length; Lateral basal process **(LBP)** is mostly knoblike, measuring 0.253mm (Table 2).

'*Tenax*' (Fig. 5B): Apical Sternal Angle (**ASA**) of inner division of an apical sternal speculate part strongly produced sterned into a sharp beak with interiorly curved apex, 0.396mm in length with little distinct emargination on apical margin. Apical Tergal Angle **(ATA)** protrude outwardly but not beaked; inner tergal surface with distinct lobe bearing 2–3 budlike fused Folliform processes (**FP**), these buds varying in length. Outer division (**OD**) is in shape of a broad acuminate leaf pointing downward with 0.603mm horizontal length; Lateral Basal Portion **(LBP)** is mostly cuboidal, measuring 0.381mm while Basal Sternal lobe (**BSL**) is slender in shape and vary in length (Table 2).

Typical - ***Variant*–I (Fig. 6A):** Apical Sternal Angle (**ASA**) of sternal speculate portion of inner division is beaked, average measuring 0.35mm along with slight curved apical margin of 0.81mm.

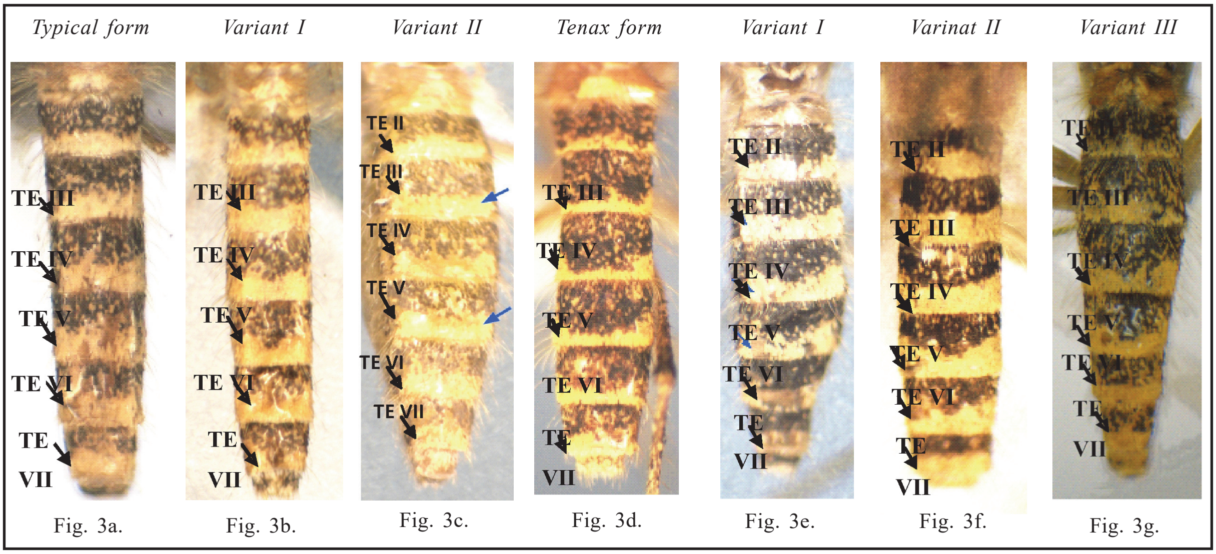
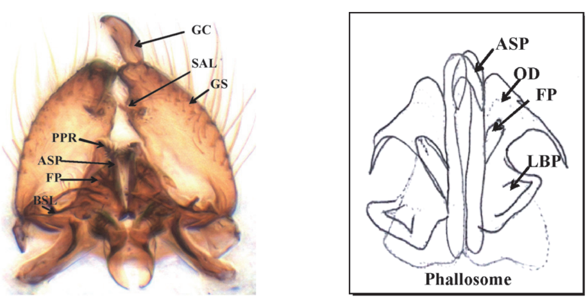
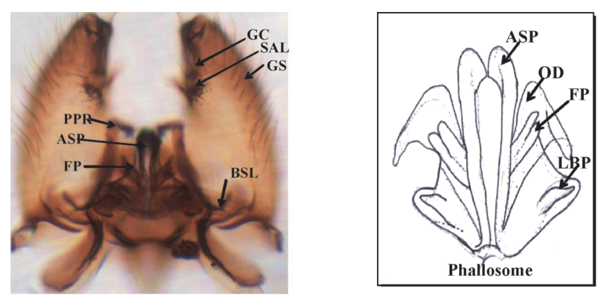


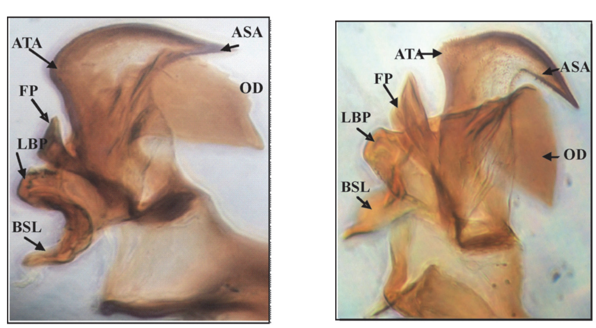
Fig. 3 Phenotype of Abdominal Terga (TE) of both '*typical* and '*tenax'* forms with its intraspecific variants of *Cx. bitaeniorhynchus*



General view
Fig. 4A. Male Genitalia of *Culex bitaeniorhychus* ('*typical'*)



General view
Fig. 4B. Male Genitalia of *Culex bitaeniorhychus* ('*tenax'*)

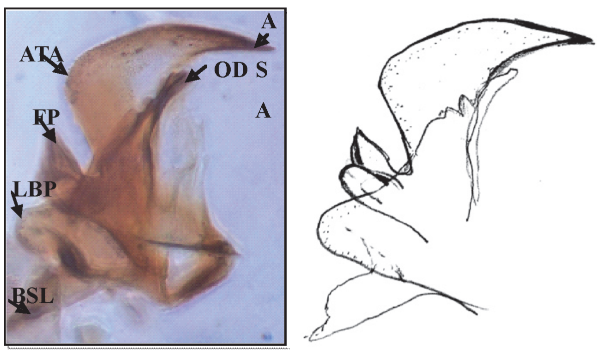


'Typical' form (Fig. 5A) '*Tenax'* form (Fig. 5B)

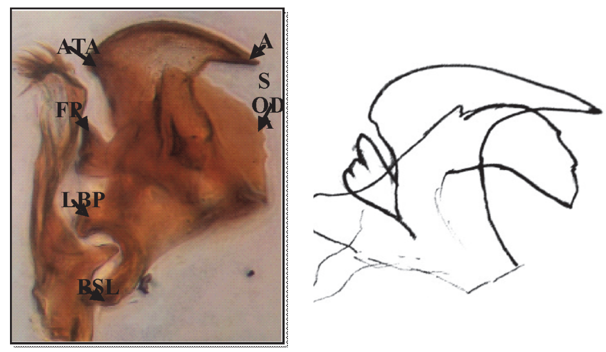
Fig. 5 Lateral view of phallosome of male genitalia

Apical Tergal angle (**ATA**) mainly blunt or obtuse in shape. The inner tergal surface with distinct lobe bearing distinct 2–3 fingerlike folliform processes (**FP**), in which basal folliform process is short (0.30mm). Outer division (**OD**) is in shape of short acuminate leaf with average 0.51mm horizontal length; Lateral basal process **(LBP)** is mostly slender, measuring 0.23mm, whereas, Basal Sternal lobe (**BSL**) is slender in shape (Table 2).

Intraspecific variations in the lateral plate of phallosome of male genitalia

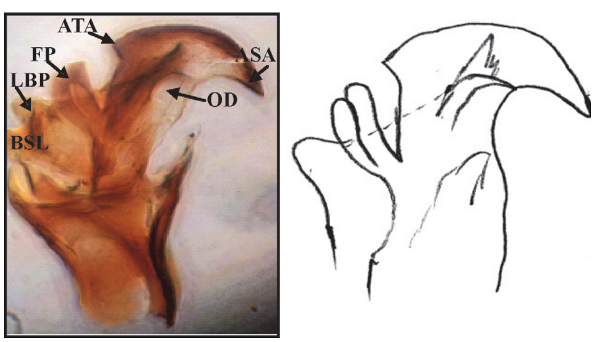


Variation I (Fig. 6A)



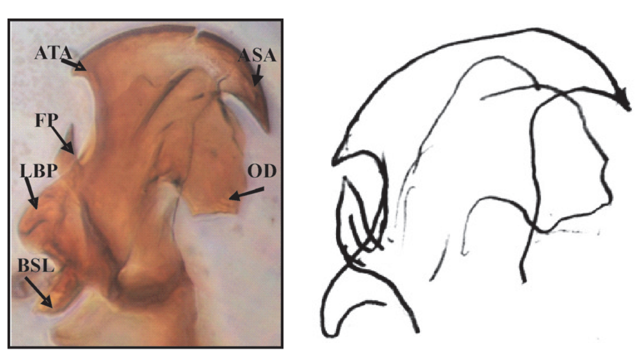
Variation II (Fig. 6B)

Fig. 6 *'typical'* form



Variation I (Fig. 7A)

Variation II (Fig. 7B)



Variation III (Fig. 7C)

Fig. 7 *'tenax'* form

Table 1a. Morphotaxonomic details of different variants in *Typical* and *Tenax* forms of *Cx. bitaeniorhynchus* Giles

Phenotype	*Typical* Form		*Tenax* Form	
	Typical	Intraspecific variations	*Tenax*	Intraspecific variations
Head (colour of erect scales on center of vertex)	Whitish or pale scales	***Variation* I** Whitish/golden pale ***Variation* II** Bronzy brown	Dark brownish to partially pale with some pale scales in center	***Variation* I** Whitish pale ***Variation* II** Dark brown ***Variation* III** Bronzy brown
Thorax (colour of scales on Scutum)	Anterior 2/3 pale, whitish, yellowish, Posterior ¼ some pale scales	***Variation* I** Golden or pale/ some whitish pale scales ***Variation* II** whole SC with Bronzy pale with white mottling	Anterior 2/3 Pale to bronzy brown Posterior ¼ few white scales	***Variation* I** Dense Whitish/Few whitish pale scales ***Variation* II** Dense brownish/ dark scales ***Variation* III** Dense pale brownish /without pale scales
Legs	Extensively speckled with black and pale scales	***Variation* I** Extensively speckled with more pale scales ***Variation* II** Lightly speckled with black and pale scales	Light to moderately speckled	***Variation* I** Light speckled with more dark scales ***Variation* II** Moderately speckled ***Variation* III** Light to moderately speckled
Wings	Extensively speckled with black and pale scales on all veins	***Variation* I** Extensively speckled with black and pale scales on all veins ***Variation* II** Lightly speckled with black and pale scales on all veins	Lightly to moderately speckled	***Variation* I** Heavily speckled with golden pale scales ***Variation* II** Heavily speckled with whitish pale scales ***Variation* III** Heavily speckled with more pale scales on all veins
Abdomen	Broad apical and basal bands on terga II-VII, tergaVIII with both basal and apical scales(Fig. 3a)	***Variation* I** Broad golden pale apical bands present on terga II-VII and apical and basal bands on VIII terga. (Fig. 3b)Black tergal area speckled with pale scales ***Variation* II** Moderatly broad whitish pale apical bands present on terga II-VII and apical and basal bands on VIII terga (Fig. 3c) Black tergal area lightly speckled with pale scales.	Terga II-IV with Pale yellowish apicolateral spots forming very narrow apical bands; terga V-VIII with narrow apical yellowish bands connecting with large yellowish apicolateral spots at sides. (Fig. 3d)	***Variation* I** Terga III-IV with whitish Pale apicolateral spots forming narrow apical bands; terga V-VIII with narrow apical palish bands connecting with large yellowish apicolateral spots at sides (Fig. 3e) ***Variation* II** Terga II-IV with Pale yellowish apicolateral spots forming broad apical bands; terga V-VIII with narrow apical yellowish bands connecting with large yellowish apicolateral spots at sides (Fig. 3f) ***Variation* III** Terga II-V with Pale yellowish apicolateral spots forming very narrow apical bands; terga V-VIII without narrow apical yellowish bands at sides (Fig. 3g)

Typical - ***Variant*–II (Fig. 6B):** Apical Sternal Angle (**ASA**) of inner division of sternal portion is beaked, measuring 0.43mm average length along with curved apical margin. Apical Tergal Angle (**ATA**) mainly smoothly curved. The inner tergal surface with distinct lobe bearing distinct 2–3 fused fingerlike folliform processes (**FP**) measuring 0.588mm average size of basal folliform process. Outer division (**OD**) is in shape of short acuminate leaf with 0.808mm horizontal length; Lateral Basal processes **(LBP)** are mostly slender with down projection, measuring 0.269mm and Basal Sternal lobe (**BSL**) is slender with down projection (Table 2).

***Tenax - Variant*–I (Fig. 7A):** Apical Sternal Angle (**ASA**) of sternal speculate portion of inner division to apex is beaked, measuring 0.395mm along with curved apical margin. Apical Tergal Angle (**ATA**) mainly sharply beaked. The inner tergal surface with distinct lobe bearing two long fused fingerlike folliform processes (**FP**), in which the basal folliform process is measuring 0.493mm. Outer division (**OD**) is in shape of short acuminate leaf with 0.598mm horizontal length; Basal Sternal lobe (**BSL**) is cuboidal in shape while, Lateral Basal processes **(LBP)** are mostly slender with down projection, measuring 0.227mm (Table 2).

***Tenax - Variant*–II (Fig. 7B):** an Apical Sternal Angle (**ASA**) of sternal speculate part of inner division to apex is sharp beaked, measuring 0.352mm along with curved apical margin. Apical Tergal Angle (**ATA**) mainly bluntly beaked. The inner tergal surface with distinct lobe bearing distinct 2–3 fused fingerlike folliform processes (**FP**), in which basal process is 0.499mm in length. Outer division (**OD**) is in shape of short acuminate leaf shaped with 0.836mm in horizontal length; Lateral Basal processes **(LBP)** is slightly oval in shape while the Basal Sternal lobe (**BSL**) are mostly slender with down projection, measuring 0.316mm (Table 2).

***Tenax - Variant*–III (Fig. 7C):** an Apical Sternal Angle (**ASA**) of sternal speculate portion of inner division to apex is sharp beaked**,** measuring 0.889mm along with curved apical margin. Apical Tergal Angle (**ATA**) mainly shortly beaked. The inner tergal surface with distinct lobe bearing distinct 2–3 fused finger shaped folliform processes (**FP**) measuring 0.481mm. Outer division (**OD**) is in shape of short acuminate leaf with 0.558mm horizontal length; Lateral Basal Processes **(LBP)** are mostly oval with down projection, measuring 0.384mm while Basal Sternal lobe (**BSL**) is slender shaped and projected downwardly (Table 2).

Table 2. Morphometric analysis of various parts of phallosome of *Cx. bitaeniorhynchus*

S.no.	Phenotypics variations	*n*	AM	ASSP	OD	FP	LBP
1.	***Typical* form**	90	0.964	0.414	0.591	0.458	0.253
2.	*variant* I	16	0.815	0.358	0.518	0.304	0.179
3.	*variant* II	6	0.918	0.432	0.888	0.588	0.269
			0.89±0.08	0.40±0.05	0.66±0.17	0.45±0.15	0.23±0.04
4.	***tenax* form**	23	0.891	0.396	0.603	0.538	0.381
5.	*variant* I	10	0.766	0.395	0.598	0.493	0.227
6.	*variant* II	5	0.969	0.352	0.836	0.499	0.316
7.	*variant* III	4	0.889	0.275	0.558	0.481	0.384
			0.87±0.11	0.35±0.08	0.64±0.11	0.50±0.06	0.32±0.08

* Length measured in mm (Mean±SD); Number of mosquito sample collected-n

One factor ANOVA along with parametric tests was performed to confirm statistical significance in morphological differences in '*typical*' as well as '*tenax*' form and their respective intraspecific variants of *Cx. bitaeniorhynchus*. The significant p value (Confidence interval of 95%) for both of these forms of *Cx. bitaeniorhynchus* has been found to be <0.05, Hence, these two forms are statistically significant and rejecting the null hypothesis and accepting the alternate hypothesis. Furthermore, pair wise comparison of each phenotypic variant along its naturally occurring forms was also done statistically in a single analysis (Table 3).

DISCUSSION

Culex bitaeniorhynchus subgroup comprises of species which are extremely similar and difficult to identify because of overlapping suites of shared phenotypic features. Previous taxonomic workers (Edward, 1922; Colless, 1959; Sirivanakarn, 1973, 1976) have studied *Cx. bitaeniorhynchus* along with different forms on the basis of striking and discontinuous variations in coloration of scales on different parts of the body. They also mentioned the presence of different variants in *Cx. bitaeniorhynchus*. Edward (1922) distinguished *Cx. bitaeniorhynchus* (type form) into three varieties like '*tenax*', '*ambiguus*', '*domesticus*' and two unnamed forms from Oriental region. Barraud (1934) extensively surveyed all parts of India and studied *Cx. bitaeniorhynchus* along with male genitalia. He indicated the presence of two or more variabilities in genitalic characters of *Cx. bitaeniorhynchus* and also mentioned three varieties i.e *var*. '*ambiguus*', *var*. '*tenax*' and one unnamed variety in the Fauna of British India. Bram (1967) reported 3 forms from Thailand i.e common or typical form, '*tenax*' form, '*ambiguus*' form and one intermediate form. After many years, Sirivanakarn (1973) recognized number of forms within *Cx. bitaeniorhynchus i.e*. '*tenax*' Theobald, '*domesticus*' Leicester, '*ambiguus*' Theobald, '*taeniarostris*' Theobald, '*sarawaki*' Theobald from Southeast Asia. In 1976, he mentioned five species under *Cx. bitaeniorhynchus* complex *viz*; *bitaeniorhynchus* (typical form), *infula*, *luzonensis* (*luzon* form), *selangorensis* (*selagor* form), *pseudosinensis* and *longicornis*. He also mentioned that all these component species show many overlapping characteristics with one another in their phenotype, so usually pose great difficulty in their correct identification. He lumped all available multiple variants like '*ambiguus*', '*tenax*' *var. 'ocellata'*, '*domesticus*', '*taeniarostris', 'infula'* and *'sarawaki'* into single species i.e. *infula.* Darsie & Pradhan (1990) found only female of '*ambiguus*' from Nepal and agreed provisionally

Table 3. Results of one-way ANOVA for comparing means of phenotypic attributes in both forms of *Cx. bitaeniorhynchus*

	***Typical* form**	**Var. I**	**Var. II**			***Tenax* form**	**Var. I**	**Var. II**	**Var. III**		
Phenotypic Attributes	**Mean± SD**	**Mean± SD**	**Mean± SD**	**F**	**Sign. (p)**	**Mean± SD**	**Mean± SD**	**Mean± SD**	**Mean± SD**	**F**	**Sign. (p)**
AM	0.96± 0.02	0.81± 0.005	0.91± 0.10	4.21	0.07	0.89± 0.16	0.7± 0.01	0.9± 0.05	0.8± 0.09	1.76	0.22
ASSP	0.41± 0.03	0.35± 0.02	0.43± 0.06	1.77	0.18	0.39± 0.08	0.3± 0.004	0.3± 0.004	0.2± 0.13	1.07	0.04
OD	0.59± 0.06	0.51± 0.03	0.88± 0.007	0.01	0.01*	0.6± 0.05	0.8± 0.03	0.83± 0.03	0.5± 0.03	41.3	0.001*
FP	0.45± 0.005	0.30± 0.002	0.58± 0.18	21.0	0.04	0.5± 0.008	0.4± 0.006	0.4± 0.006	0.4± 0.14	0.23	0.726
LBP	0.25± 0.01	0.17± 0.009	0.26± 0.02	16.7	0.02*	0.38± 0.89	0.3± 0.005	0.3± 0.005	0.3± 0.08	2.23	0.016*

with Sirivanakarn about '*ambiguus'* a synonym of *infula* and left it pending for further studies.

However, during present investigations, both male and female of 'typical' and '*tenax'* has been found and studied. After detailed morphotaxonimic studies and careful observations on important characteristics of male genitalia it has been concluded that '*tenax'* totally resemble with the '*tenax'* mentioned by Sirivanakarn (1973), not with '*infula'* as mentioned by him in 1976. According to him, apical spiculate portion of inner division of phallosome of '*infula'* is strongly pigmented and is with deep distinct emargination proximad of apical tergal angle. The folliform processes on inner tergal lobes are 4–5 in number. However, in '*tenax'* it has been observed that the interiorly emargination on apical sternal speculate part is not much deeply distinct and apical sternal angle of inner division strongly produced sterned into sharp beak with a little pigmentation. The folliform processes on inner tergal lobes are 2–3 in number. Moreover, outer divisions of phallosome is lumped inferiorly in '*infula'* while, it is in the shape of a broad acuminate leaf which points downward with distinct horizontal length in '*tenax'*. The basal sternal process of proctiger in '*infula'* is relatively stronger (Sirivanakarn, 1973, 1976) but, in '*tenax'* it is usually slender in shape. Hence, '*tenax'* which is strikingly different from '*infula'* should not be synonymised with '*infula'*.

The present study revealed the occurrence of *Cx. bitaeniorhynchus* in Chandigarh as '*typical'* and '*tenax'* forms. The '*tenax'* form was synonymised provisionally with species '*infula'* by earlier workers. But, in the present exploration, it has been perceived that '*tenax'* is incredibly different from '*infula'* in morphology and male genitalia and hence, should not be synonymised with species name '*infula'*. It is also evident from the statistical analysis which showed significant difference between these two forms.

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Ovicidal and larval repellent efficacy of *Tagetes erecta* Linn. on *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae)

S. Sahina, M.I. Zahir Hussain* and R. Balasubramanain#

Sadakathullah Appa College (autonomous), Rahmath nagar, Tirunelveli 627011, Tamil Nadu , India; #National Institute of Virology - Kerala unit, Alappuzha 688005, Kerala, India.
Email: sahinasmail@gmail.com; mizahirhussain@gmail.com; balasniv@gmail.com

ABSTRACT: A study was undertaken on the ovicidal and larval repellent activity of *Tagetes erecta* leaf and flower extracts on *Rhipicephalus sanguineus* (Latreille, 1806), an important tick species in the world from an economic and medical point of view. Ethanol and methanol extracted plant products tested against the eggs and larvae of *R. sanguineus* indicated that the ethanol extract of flower had maximum ovicidal activity (86.1%), followed by the ethanol extract of leaf (75%) at 25 mg ml^{-1} concentration. In all analyses, the homogeneity of variance was significant. The probit analysis clearly indicated that the ethanol extract of the flower has a higher ability to kill the eggs. In the case of larval repellency tested, both extracts of leaf showed the highest repellency (83%) at 2.5 mg ml^{-1}. Significant tick repellency (> 90%) was found in both methanol and ethanol extracts of flower at 2.5 mg ml^{-1}. GC-MS analysis of extracts revealed the presence of bioactive insecticidal compounds such as yangambin, cyclohexane and neophytadine.

KEYWORDS: Tick, ovicidal, larvicidal, repellency, marigold, bioactive compounds

INTRODUCTION

Ticks (Acari: Ixodidae) are the obligate ectoparasites of animals and are responsible for the transmission of numerous infectious agents such as pathogens to vertebrates, including viruses, bacteria, protozoa, and helminths (De la Fuente *et al*., 2008). In recent studies, tick and tick-borne diseases being much concentrated because of their increasing incidence and significant harm to livestock and human health (Balasubramanian *et al.*, 2019). *Rhipicephalus sanguineus* (Latreille, 1806), brown dog tick is the most important tick species in the world as a vector of various disease-causing pathogens like *Coxiella burnetti, Rickettsia conorii* and *R. rickettsii* for animals as well as for human beings (Sonenshine and Roe, 2014). In India, the causative pathogens of Indian tick typhus (ITT), a type of rickettsial spotted fever (similar to Rocky Mountain spotted fever), and Babesiosis are transmitted by *R. sanguineus* (Srikant Ghosh and Gaurav Nagar, 2014; Brites-Neto *et al*., 2015). Studies with an emphasis on tick controls are limited to India. Chemical

* Author for correspondence

acaricides like amitraz, synthetic pyrethroids used to control resulted in the development of resistance to the ticks (Eiden *et al*., 2015; Rodriguez-vivas *et al*., 2017). Plant-derived natural acaricides are the suitable alternative for chemical acaricides with minimum toxicity, high rate biodegradation and less resistance development (Quadros *et al*., 2020). Although many plant species have been traditionally used to control ticks (Adenubi *et al.,* 2016), the efficacy of extracts of many of the plant species have not been investigated and validated. The plant *Tagetes erecta Linn.* belonging to the *Asteraceae* family, commonly known as marigold, is commonly cultivated in India and has acaricidal properties (Wanzala *et al*., 2014; Fabrick *et al*., 2020). Hence a study was undertaken on the ovicidal and larval repellent activity of ethanol as well as methanol extracts of *T. erecta* leaf and flower on *R. sanguineus*.

MATERIALS AND METHODS

From the blood-fed adult ticks collected from cattle and naturally infected dogs, *R. sanguineus* species ticks were selected after the identification by morphological identification key (Hans *et al*., 2001). Ticks were placed in a plastic container (7 X 5 cm) capped with a piece of cotton cloth tubes were placed in an incubator (28 ±1°C and RH ≥ 80%). Freshly laid eggs and subsequent larva were used for the experiments.

Marigold plant at the full bloom stage was confirmed as *T. erecta* from the faculty of Department of Biological science, Gandhigram University, Dindigul, Tamil Nadu. The leaves and flowers of the *T. erecta* plants were washed and dried at room temperature (25–30°C) and powdered using an electric grinder. The powdered flower and leaf are stored in a sealed bottle at room temperature. About 75–100 g of dried plant powder was weighed and kept in a thimble chamber of the soxhlet apparatus. Ethanol and methanol extraction of each sample was carried out in 75" 85 cycles at a temperature ranging from 40–55°C, and the extract was concentrated by evaporation at 40-55°C and then dried and kept at 4°C for the bioassays.

The phytochemicals in the extracts were identified as per Harborne (1998) and Kokate (2001). The analysis of the flower and leaf extracts was performed using a gas chromatograph coupled to a mass spectrometer (GC–MS) equipped with an auto-injector and a fused-silica capillary column. Helium was used as the carrier gas at a flow rate of 1.2 ml per minute. Injector and detector temperatures were set at 250°C and 280°C, respectively. Column temperature was set to 60°C for 5 minutes, then gradually increased to 160°C at 4°C for one minute and finally increased to 270°C at 15°C for one minute.

A stock solution of plant extracts was prepared in dimethyl sulpho oxide (DMSO). From the stock solution, concentrations of 5, 10, 15, 20, and 25 mg ml^{-1} meant for ovicidal repellency bioassay and concentrations of 0.5, 1, 1.5, 2 and 2.5 mg ml^{-1} for larval repellency bioassay were prepared.

Ovicidal assay: Twenty numbers of eggs were placed in glass vials (5cm x 2cm) with filter paper at the bottom and topically sprayed with 5 ml of different extracts with concentrations of 5, 10, 15, 20 and 25 mg ml^{-1}. Control eggs were treated with one per cent DMSO only. Three replicates were maintained for each treatment, and the experiment was conducted in an incubator (28 ± 1°C and RH ≥ 80%) and regularly observed until hatching began. The hatched larvae were separated every day from the unhatched eggs and observed for two more weeks before they were declared unhatched and dead. The ovicidal activity (%) was assessed by the following formula:

$$\frac{\text{Number of unhatched eggs X 100}}{\text{Total number of eggs introduced}}$$

Repellency Bioassay: The experiment was carried out in a test model with a funnel based on the combination of ambushing and hunting behavior of ticks. Test and control Whatman No.1filter papers (2.5 × 2.5 cm) were treated with 5 ml of different concentrations (0.5, 1, 1.5, 2 and 2.5 mg ml^{-1}) of sample solutions and air-dried for one hour. The control filter paper was impregnated with one per cent DMSO. The treated and control paper

was placed in the middle of the tail tube of the funnel (5 × 0.5 cm). Twenty larval ticks were introduced on a base plate (7 × 1.5 cm). Ticks that were climbed on the upper part of the filter paper were considered not repelled, and those on the bottom of the filter paper, naked part of the apparatus, and on the base plate were considered repelled. Each experiment was repeated three times. The percentage repellency was calculated as:

$$100 - \frac{\{\text{Mean no. of ticks in test} \quad \text{X } 100\}}{\{\text{Mean no. of ticks in control}\}}$$

Statistical analysis: Probit analysis (EPA 2006) was used to analyse the ovicidal and larval repellency percentage with the calculation of confidence interval (CI) of the mean number of ticks repelled as well as the egg mortality by the treatment. Each replication was considered independently. Statistical significance on dose response with each concentration was determined by ANOVA. All significant levels are set at $P<0.05$. SPSS windows version IBM 20 was used for data analysis.

RESULTS AND DISCUSSION

The leaves and flowers of *T. erecta* in ethanol (EtOH) and methanol (MeOH) extracts were analysed for their phytochemical contents. Phytochemicals such as alkaloids, flavonoids, saponins, tannin, cardiac glycosides, and terpenoids were found. Tannins were found strong positive in both the extracts of leaves and flowers, followed by alkaloids and flavonoids. Cardiac glycosides, terpenoids and saponins were found in EtOH flower extracts (Table 1).

The bioactive compounds present in the leaves and flowers extracts, identification and characterization were based on their retention time in an HP-5MS column (Table 2, 3). Based on abundance, the three major compounds present in the MeOH extract of leaf were 1-butanol, 3-methyl-formate (39.10%), benzofuran (8.10%) and octyl-beta-D-glucopyranoside (7.15%). The EtOH leaf extract contained yangambin (69.44%) followed by alpha-tocopherol (14.65%) and neophytadine (4.73%) as major compounds. Diethyl phthalate (27.98%), Alpha D-Glucopyranose (19.63%) and 4H-Pyran (5.79%) as three major compounds in the MeOH extract of flower. Major compounds found in EtOH extracts of the flower are the yangambin (30.81%), 3H-Forofuran (29.78%), and Beta D-glucopyranose (10.98%).

Ovicidal Activity: Among the four extracts tested, the EtOH extract of flower recorded the highest ovicidal activity (86.1%), followed by the EtOH extract of leaf (75%) at 25mg ml^{-1}. The MeOH extracts of both leaf and flower showed 65 and 69.3 per cent respectively, ovicidal activity at 25 mg ml^{-1} (Table 4). The homogeneity of variance was significant at all the analyses and the ANOVA was significant (P value <0.05). The R^2 indicate that EtOH extracts of flowers had maximum

Table1. Phytochemical compounds in methanol and ethanol extracts of *T. erecta* leaves and flower

Phytochemicals	Test	Leaf		Flower	
		MeOH	EtOH	MeOH	EtOH
Alkaloid	Wagners	—	+++	—	+++
Flavonoid	Lead acetate	—	+++	—	+++
Saponin	Froth	+	—	+++	+++
Tannin	Ferric chloride	+++	+++	+++	+++
Cardiacglycoside	Keller-Killianis	—	—	—	++
Terpenoid	Salkowski	—	—	—	++

+ mild positive ++ Average — Negative, +++ strong positive

Table 2. Biochemical compounds in methanol and ethanol extracts of *T. erecta* leaves by GC-MS analysis

Methanol Extract			**Ethanol Extract**		
Compound	R T*	%	Compound	R T*	%
Cyclohexanamine,N-3-butenyl-N-methyl-	4.158	1.54	1-Butanol,3-methyl-formate	4.496	1.36
1-Butanol,3-methyl-formate	4.604	39.10	2-cyclohexen-1-one,3-methyl-6-	8.400	1.99
Aceticacid, pentylester	5.299	2.10	Neophytadiene	28.774	4.73
4h-pyran-4-one,2,3-dihydro-3, 5-dihydroxy	5.545	5.02	phytolisomer	35.909	2.61
benzofuran,2,3-dihydro-	7.244	8.10	hexadecanoicacid	43.255	1.01
benzaldehyde,2-hydroxy-6methyl	14.458	2.01	beta.-tocopherol	44.852	0.51
octyl-.beta.-d-glucopyranoside	20.866	7.15	methyl(z)-5,11,14,17-eicosatetraenoate	46.262	1.16
nonylamine,n,n-di(allyl)-	22.575	6.55	28-norolean-17-en-3one	46.523	1.59
Hexylamine,N,N-di(allyl)-	25.151	7.06	yangambin	47.643	69.44
Muco-Inositol	31.750	4.74	alpha.-Tocopherol-beta-D-mannoside	48.843	14.65

* Retention time

Table 3. Biochemical compounds in methanol and ethanol extracts of *T. erecta* flowers by GC-MS analysis

Methanol Extract			**Ethanol Extract**		
Compound	R T*	%	Compound	R T*	%
Thymine	4.175	4.18	1-Butanol,3-methyl-formate	4.160	0.85
2-Butanone,4-hydroxy-3-methyl-	5.25	4.38	2-Butanone,4-hydroxy-3-methyl-	4.512	1.93
4H-Pyran-4-one,2,3-dihydro-3, 5-dihydroxy-	5.551	5.79	4H-Pyran-4-one,2,3-dihydro-3, 5-dihydroxy-	5.539	1.20
Ketone,methyl2-methyl-1, 3-oxothiolan	7.838	3.27	2-Pyrrolidineaceticacid	7.027	0.54
beta.-Alanine,N-acryloyl-, isobutylester	8.85	2.52	Phenol, 2,6-dimethoxy-	11.225	4.14
Phenol, 2,6-dimethoxy-	11.225	7.46	beta.-D-Glucopyranose,4-O-. beta.-D-galact	19.853	0.69
Diethyl Phthalate	19.833	27.9	Nonylamine,N,N-di(allyl)-	25.149	0.51
Hexylamine,N,N-di(allyl)-	25.147	2.99	n-Hexadecanoicacid	32.339	0.28
n-Hexadecanoicacid	31.462	0.68	Stigmasta-5,20(22)-Dien-3-OL	43.998	3.32
.alpha.-D-Glucopyranose,4-O-. beta.-D-galac	35.951	19.6	Yangambin	45.925	30.81
(2,3,5,6-Tetrafluorophenyl)methyl 3-(2,2-dic	38.20	2.65	1h,3h-furo[3,4-c]furan,tetrahyd	47.264	29.78
.gamma.-Sitosterol	47.729	2.82	.gamma.-Sitosterol	47.86	4.74

* Retention time

Table 4. Effect of methanol and ethanol extracts of *T. erecta* on *R. sanguineus* eggs (% mortality ± SE)

Conc. ($mg\ ml^{-1}$)	Leaf		Flower	
	MeOH	EtOH	MeOH	EtOH
5	12.50±0.12	18.33±0.18	21.50±0.13	24.50±0.14
10	13.33±0.12	20.33±0.14	32.10±0.12	39.20±0.13
15	21.66±0.14	25.00±0.18	39.60±0.12	58.20±0.12
20	58.33±0.13	46.60±0.13	62.70±0.18	75.50±0.12
25	65.00±0.13	75.00±0.12	69.30±0.18	86.10±0.10

Table 5. Statistical analysis of ovicidal activity of methanol and ethanol extracts of *T. erecta* against *R. sanguineus* eggs

Extract	LC_{50}	LC_{90}	R^2	df	P value*	Upper Cl	Lower Cl	Regression
Leaf–MeOH	20.41	72.44	0.79	4	0.04	4.38	0.18	Y=2.00-2.28x
Leaf–EtOH	19.49	89.12	0.67	4	0.05	4.37	-0.50	Y=2.50-1.93x
Flower–MeOH	15.84	77.62	0.86	4	0.02	3.17	0.50	Y=2.79-1.84x
Flower-EtOH	10.71	34.67	0.94	4	0.005	3.58	1.38	Y=2.44-2.48x

*P value is significant at 0.05 level; MeOH – Methanol; EtOH – Ethanol; LC50 - Lethal concentration at 50%; LC90 - Lethal concentration at 90%; R^2 - Proportion of the variance; df – degree of freedom; Upper Cl – Upper confidance interval at 95%; Lower Cl – Lower confidence interval at 95%.

Table 6. *R. sanguineus* larval repellency (%) in different concentrations of methanol and ethanol extracts of *T.erecta*

Conc. ($mg\ ml^{-1}$)	Leaf		Flower	
	MeOH	EtOH	MeOH	EtOH
0.50	12.50	25.00	21.40	41.60
1.00	33.30	67.50	75.00	78.50
1.50	41.20	75.00	87.50	92.80
2.00	63.30	87.50	91.80	99.00
2.50	83.00	86.10	96.80	99.00
Lower CI	1.32	1.88	2.64	2.77
Upper CI	4.16	3.68	4.75	4.93

ovicidal activity of (R^2=0.94). MeOH extract of leaf required higher concentration (20.41 $mg\ ml^{-1}$) for 50 per cent egg mortality, whereas EtOH extract of flower required only 10.71 $mg\ ml^{-1}$ for 50 per cent and 34.6 $mg\ ml^{-1}$ for 90 per cent egg mortality (Table 5). The probit analysis clearly indicates that the EtOH flower extract has maximum potential to kill the eggs of *R. sanguineus.* The toxicity values of treated extracts of *T. erecta* based on LC_{50} values could be arranged in descending order as

follows: EtOH flower extract > MeOH flower extract > EtOH leaf extract > MeOH leaf extract. The control eggs treated with the one per cent DMSO recorded with zero mortality.

Larval Repellency: All tested larval ticks showed repellency against all extracts tested except control. The larval repellency observed in MeOH extract of leaf ranged from 12.5 per cent in the lowest 0.5 mg ml^{-1} to 83 per cent in the highest 2.5 mg ml^{-1} concentration. EtOH extract of leaf larval repellency ranged from 25 in 0.5 mg ml^{-1} to 83 per cent 2.5 mg ml^{-1} concentration (Table 6). The repelled larval ticks were found in the naked regions of the test apparatus or resting on the platform. There was a significant (R^2= 0.97, P-value = 0.001) dose – tick repellency response relationship. Tick repellency (> 90%) was found in both MeOH and EtOH flower extracts at 2 mg ml^{-1} concentration producing an RC_{50} of 0.77 and 0.58 per cent respectively (Fig. 1).

The leaves and flowers of *T. erecta* have been used in India and other South East Asian countries traditional medicine to treat various pain and inflammatory conditions (Singh *et al*., 2020; Rahman *et al*., 2020). The current study establishes the ovicidal and anti-larval properties of the EtOH and MeOH leaf and flower extract of *T. erecta* against *R. sanguineus*. The acaricidal activity of the *T. erecta* extracts in our study is consistent with results from other studies. However, direct comparisons are difficult to make as no other study has evaluated the relationship between the extract concentrations and the percentage of ticks killed. Even though the exact acaricidal mechanisms are yet to be established, it is possible that *T. erecta* may act through the inhibition of the release and/or action of repellent mediators (e.g., Alkaloids, flavonoids, saponins, and tannins) since it inhibited egg hatching and larval repellency (Ravikumar, 2010; Vijay *et al*., 2013). The egg mortality increased as extract concentration increased, and more than 50% mortality was induced by all the plant extracts at 20mg ml^{-1} concentration. Politi *et al.*(2012) reported that the 70 percent of EtOH extract of aerial parts of *T. patula* reduced egg laying by 21.5 per cent and eliminated 99.78 percent of the larvae of *R. sanguineus*. However, in our study, the EtOH extracts of flower produce 39.2 per cent mortality in 10mg ml^{-1} concentration. Furthermore, different *Tagetes* spp., extracts have also been shown to significantly kill various kinds of insect pests such as stored product beetles, mosquitoes and armyworms (Nikkon *et al*., 2011; Nchu *et al*., 2012; Politi *et al*., 2012).

The presence of alcoholic sugar xylitol, Butanol, 3-methyl formate, cyclohexane and neophytadiene in the leaf of the MeOH and EtOH extracts may account, at least in part, for the observed insecticidal and medicinal effects (Puterka *et al.,* 2003; Barakat,

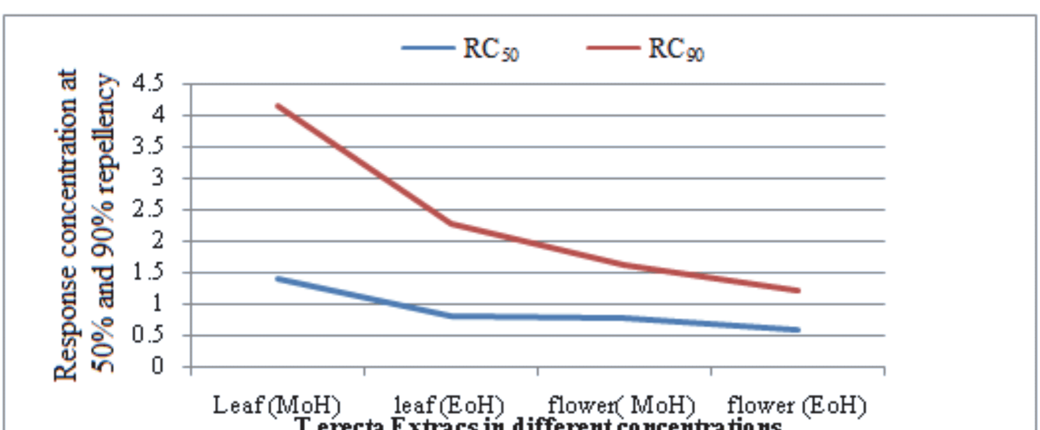

Fig. 1 Relationship between *R. sanguineus* larval repellency (RC_{50} & RC_{90}) and extracts of *T. erecta* in the bioassay

2011; Caceres *et al*., 2015; Edwin and King, 2017; Etify *et al*., 2017). The chemical compound yangambin was found highest percentage in both EtOH extracts of leaf and flower extracts. Several studies have shown that yangambin inhibits postembryonic development, morphological alteration, and oviposition reduction in harmful insect pests (Marise *et al*., 2007). The extract of *T. erecta* leaf and flower showed repellent effects on larval ticks at all concentrations tested with RC_{50} of 0.58% to 1.4%w/v. Elango and Rahuman (2011) and Vijay *et al*. (2013) reported 70 per cent acaricidal activity for *Haemaphysalis bispinosa* and 77 per cent larvicidal activity for *R. microplus* in MeOH extracts of *T.erecta* flowers. The current results indicate that the ethanol extracts of the *T.erecta* flower were more effective in exhibiting the repellent action against the larval ticks tested. The present study clearly establishes the acaricidal properties of the leaf and flower extract of *T. erecta*. EtOH extracts of this plant may be used as a source of anti-tick agents. However, further studies are needed to further elucidate the efficacy of the identified compounds in EtOH extracts of leaf and flower.

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Scanning electron microscopy of cuticular sensory structures on the legs of *Micronecta haliploides* (Horvath, 1904) and *Hydrometra greeni* (Kirkaldy, 1898) (Hemiptera: Heteroptera)

Anupama Saha and Susmita Gupta*
Department of Ecology and Environmental Science, Assam University, Silchar 788011, Assam, India. Email: saha.anupama19@gmail.com; susmita.au@gmail.com

ABSTRACT: Structures on the legs of two hemipteran bugs, *Micronecta haliploides* (Horvath, 1904) and *Hydrometra greeni* (Kirkaldy, 1898), belonging to family Micronectidae and Hydrometridae of two infra orders Nepomorpha and Gerromorpha, respectively were investigated using scanning electron microscopy. Both species have a distinctive leg structure bearing specialised cuticular sensory structures. In the study, the sensilla were classified into five basic types: sensilla trichoidea, sensilla basiconidea, sensilla placoidea, porous circular sensilla and sensilla bell mouthed. These sensilla were further differentiated on the basis of shape, size, number, flexibility and type of socket attached. A total of 26 types of sensilla in the legs of these two species were observed. *M. haliploides* showed 18 types of sensory structures and *H. greeni* 8 types. A specific morphological structure of the porous circular sensilla was observed and found to be unique.

KEYWORDS: Aquatic and semiaquatic bugs, sensilla, types, Micronectidae, Hydrometridae

INTRODUCTION

Insect cuticle provides mechanical support, protection against loss of water, infections and acts as a barrier between insect body and its environment (Imms, 1957; Moussian, 2010). Scanning electron microscope (SEM) is used to study the surface morphology of the biological samples over a large range of magnification (Zhou and Li, 2015), especially for studying the fine structure on the surface of insect epicuticle. The rigid cuticle possesses specialized cuticular structures that respond to vary minute energy change or stimuli like touch, light, temperature, pressure, humidity, chemical and mechanical forces (Brożek and Bourgoin, 2013). These are the primary sensory structures of insects that provide information about the internal and external environment. The cuticular structures are of different shapes and sizes, which are classified into four basic types: hair, campaniform, chordotonal and slit sensilla (French and Torkkeli, 2009). Aquatic insects of the order Hemiptera, have successfully adapted themselves in a variety of habitat types that include not only the lotic and lentic systems but also water filled tree holes, bamboo internode cavities and even pitcher plant (Kovac and Yang, 2000). They have both economic and ecological significance in an aquatic ecosystem (Miura and Takahashi, 1988; Papacek, 2001). The freshwater

* Author for correspondence

systems of north east India are mostly dominated by these true bugs, of Nepomorpha and Gerromorpha (Choudhury and Gupta, 2015; Hazarika and Goswami, 2010; Purkayastha and Gupta, 2015; Saha and Gupta, 2015, 2018; Takhelmayum and Gupta, 2011). *Micronecta haliploides* (Horvath, 1904) (Nepomorpha: Micronectidae) is true aquatic bug that live submerged in water. Not much is known about their feeding habits but are characterised as organic scrapers (Hadicke *et al.,* 2017) and their modified scoop-like fore tarsi (palae) helps them in scrapping. On the other hand, *Hydrometra greeni* (Kirkaldy, 1898) (Gerromorpha: Hydrometridae) is a semiaquatic living on the water surface and possess elongated legs, and known as "classic sit-and-wait predators" as they can wait static for long time until the prey comes closer. It is also a scavenger (Maier, 1977).

Studies on the sensory structures of aquatic and semi aquatic Hemiptera using SEM are mainly restricted to the mouth parts of Nepomorpha (Brożek, 2013) and Gerromorpha (Brożek and Zettel, 2014), antennal sensilla (Nowiñska *et al.* 2020) pala (Furth *et al.*, 1978) and abdominal terminalia of Corixidae (Tinerella, 2006), plastron respiration in Naucoridae (Parsons and Hewson, 1974), legs of Gerridae, Hebridae and Veliidae (Nowiñska and Brożek, 2017). For Hydrometridae studies were restricted to the anteclypeus and of males and females terminalia (Gapud *et al.*, 2002). A study was undertaken to explore the sensory structures of the legs of *M. haliploides* and *H. greeni* which occupy different habitats with the help SEM.

MATERIALS AND METHODS

Aquatic bugs, *M. haliploides* and *H. greeni* were collected (during January, 2013 to December 2014) from the two major lentic fresh water systems of Barak Valley region of southern Assam, namely, Bakhri Haor (24^{o} 49' 47.2'' N and 92^{o} 36' 51.3'' E) located in Hailakandi district and Sonebeel (24^{o} 41' 116'' N and 92^{o} 25' 532'' E) located in Karimganj district. Identification of samples were carried out under Motic Stereoscopic Zoom Trinocular microscope, using standard keys (Bal and Basu, 2004; Basu *et al.*, 2015; Jehamalar and Chandra, 2014; Nieser, 2002; Yang and Zettle, 2005). Identified samples were fixed for two hours in 2.5 per cent glutaraldehyde buffered with 0.1molar sodium-cacodylate. Samples were washed in buffer properly and post fixed in one per cent osmiumtetroxide for two hours. This was then followed by dehydration in an ascending concentrations of acetone (50–100%) and drying in critical point drier (Gupta and Gupta, 2004; Barman and Gupta, 2015). The specimens were mounted on aluminium stubs and metalized with gold using a sputter coating device. Then photomicrographs were taken by observing in JSM 6360 Scanning Electron Microscope (JEOL, Japan). From the SEM images, sensory structures on the legs of the two species were classified following studies of Nowiñska and Brożek (2017).

RESULTS AND DISCUSSION

Legs of *M. haliploides* (Fig. 1a) show numerous sensilla. All the three pairs of fore, mid and hind coxa are covered by thin, pointed, pliable hair-like sensilla trichoidea (ST1) (Fig. 1b). Length of these sensory hairs are much less than 20µm. The fore femur and tibia of this species are bare (Fig. 1c). The pala are devoid of palar pegs and its margin is aligned with thick and long socketed sensilla trichoidea (ST2) (Fig. 1d). These sensilla are also pointed, pliable, hair like but are much longer (more than 50µm in length) with thicker base. These sensilla are supported on collar-like flexible socket at its base. Ventral side of pala (Fig. 1e) shows some hook shaped sensilla trichoidea3 (ST3) and some long non-socketed sensilla trichoidea4 (ST4). ST3 is a curved sensilla, with a hook like structure on its tip. It is almost 10µm in length and is embedded on a flexible socket without a collar. ST4 is a much slender pointed, pliable, hair-like sensilla alongside ST3. It is more than 50µm in length, embedded on a flexible socked without collar. Pala on its dorsal side bears a large, sensilla placoidea (SP) (Fig. 1f). This sensilla is oval shaped depression on the dorsal side of pala.

Mid femur shows scally cuticle with pointed tipped, straight and stout sensilla basiconidea1 (SB1). The sensilla is more than 10µm in length and embedded

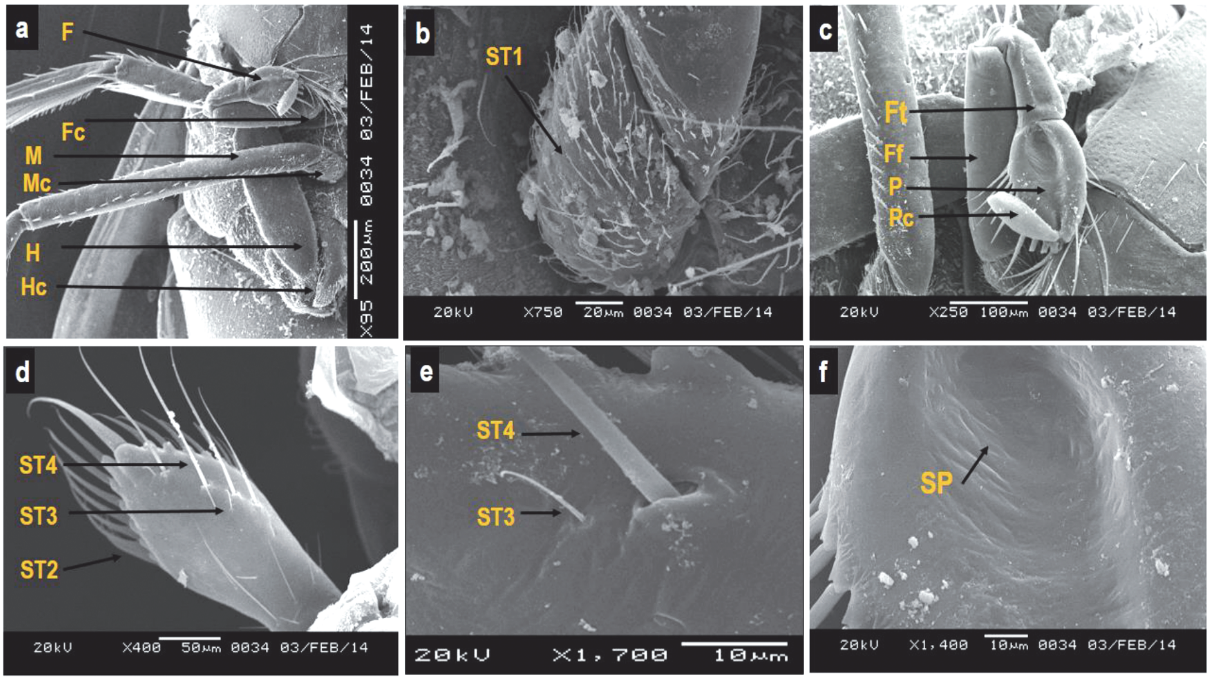

Fig.1 *Micronecta haliploides* :a) Showing fore leg (F), mid leg (M), and hind leg (H) and fore coxa (Fc), mid coxa (Mc) and hind coxa (Hc). b) The fore coxa with Sensilla trichoidea1 (ST1). c) Parts of fore leg showing the fore femur (Ff), fore tibia (Ft), pala (P) and Palar Claw (Pc). d) Showing the ventral side of pala with its margin with sensilla trichoidea2 (ST2). Pala possessing some hook shaped sensilla trichoidea3 (ST3) and some sensilla trichoidea4 (ST4). e) Enlarged view of sensilla ST3 and flexible socket of ST4. f) Enlarged view of sensilla Placoidea (SP) on the dorsal side of the pala.

on a thick flexible base without collar. In between these structures, several circular multi-porous sensilla (Cs) are seen (Fig. 2a, b). The sensilla are devoid of any hairy forms and each structure is found to be composed of about 30 to 35 pores and slits. Each sensilla is about 10- 20µm apart from each other. The mid tibia possess thin and long sensilla trichoidea 5 (ST5) with a broad raised socket. Along with it some ribbed, stout, socketed sensilla basiconidea 2 (SB2) uniformly tapered till the tip with broad base (Fig. 2c). Some sensilla SB2 are also found on the intersegmental region of tibia and tarsus (Fig. 2d). On the mid tarsus (Fig. 3a), a column of thick, bifurcated, pointed tipped sensilla basiconidea3 (SB3) are seen embedded on non-flexible socket. A small, curved sensilla trichoidea6 (ST6) similar to ST3 with curved tip embedded on a flexible socket is seen. Unlike ST3, ST6 possess a collar at its base and it is not surrounded by any other sensory structure. On the other side of SB3, a row of long, very thin hair like sensilla trichoidea7 (ST7) are also seen aligned on the tibia and tarsus (Fig. 3b). These are pliable and are embedded on non-flexible socket. At the base of the claw multi layered folded cuticular structure (FS) resembling a fish gill is observed in a groove. This structure is lined by small thick, tooth-like sensilla basiconidea4 (SB4) (Fig. 3c). Near the base of the claw a single, long, curved sensilla trichoidea3 (ST3) is noted possessing a flexible socket without collar.

Hind femur (Fig. 3d) shows broad based, relatively stout, pointed and non-flexible sensilla trichoidea8 (ST8) with the inner edge aligned by large sized thin sensilla trichoidea7 (ST7). The joint of hind femur and tibia possess small, tooth like stout sensilla basiconidea4 (SB4) and strong, thick, elongated digitiform, sensilla basiconidea5 (SB5). The hind tarsus and tibia are flat, paddle shaped and fringed with various types of sensilla supported by long

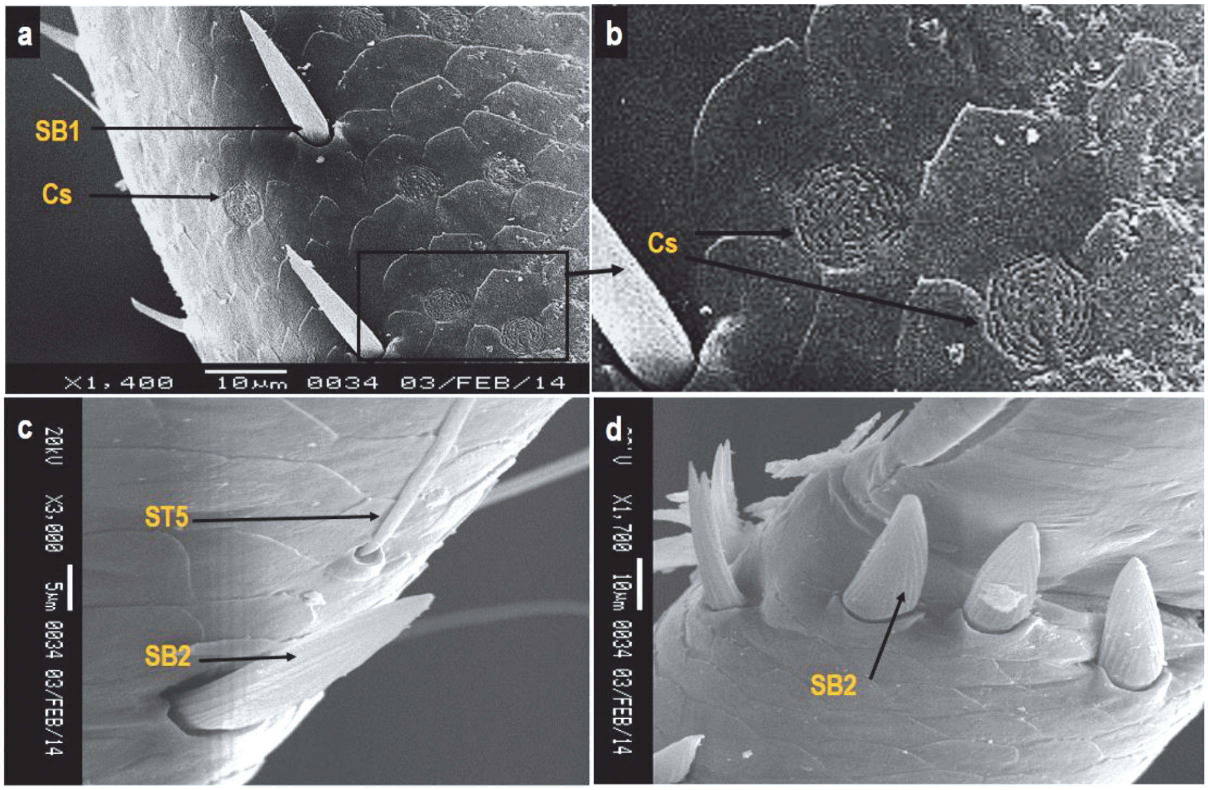

Fig.2 *Micronecta haliploides*: a) Mid femur with sensilla basiconidea1 (SB1) and Circular multi-porous sensilla (Cs) on the scales. b) Enlarged view of Cs. c) Sensilla trichoidea5 (ST5) and flexible socketed sensilla basiconidea2 (SB2) on the tibia of mid-leg. h) More similar sensilla basiconidea (SB2) on the intersegmental area of mid tibia and tarsus.

swimming hairs like sensilla trichoidea7 (ST7) (Fig. 3e). Some paint brush shaped, flat sensilla trichoidea9 (ST9) are found aligned along with the sensilla trichoidea (ST7) and on the dorsal edges ST8 sensilla (Fig. 3f). Intersegmental area of these last two segments are also lined by (SB5) (Fig. 3g). The tip of the hind tarsus also possesses some sensilla basiconidea5 (SB5) surrounding few thin, round, smooth, relatively short digitiform sensilla basiconidea6 (SB6) embedded on well-built socket (Fig. 3h).

The three pairs of legs of *H. greeni* (Fig. 4 and 5), differed in the types of sensilla and their numbers. A thorn like, non flexible socketed sensilla trichoidea10 (ST10) is seen on the bare cuticle of fore, mid and hind coxae. Near to the intersegmental region of coxa and femur a bell mouthed sensilla (BS) is seen. BS is a conical shaped, uni-porous sensilla. The pit is at the centre enclosed inside an undulating cuticular cone. On the anterior part of the fore femur, some small, thick, sensilla trichoidea11 (ST11) are aligned in a single line (Fig. 4d) whereas on the middle part of fore femur along with the ST11, relatively thinner, spine like sensilla trichoidea12 (ST12) are seen (Fig. 4e). The number and rows of ST12 are found to increase towards the apical part of the femur (Fig. 4f). On the fore tibia (Fig. 4g), density of sensilla trichoidea (ST12) intensifies superimposing the cuticle and the ST11 sensilla trichoidea. The intersegmental area between fore-tibia and tarsus shows an undulating surface (Fig. 4h) that possess relatively thick, spine like sensilla basiconidea7 (SB7). Unlike SB5, this sensilla do not bear a collar on its flexible socket (Fig. 4i). The basal part of the mid femur has sensilla ST11 (Fig. 5 a, b), whereas the apical part has an array of pliable, thin smoothly curved sensilla

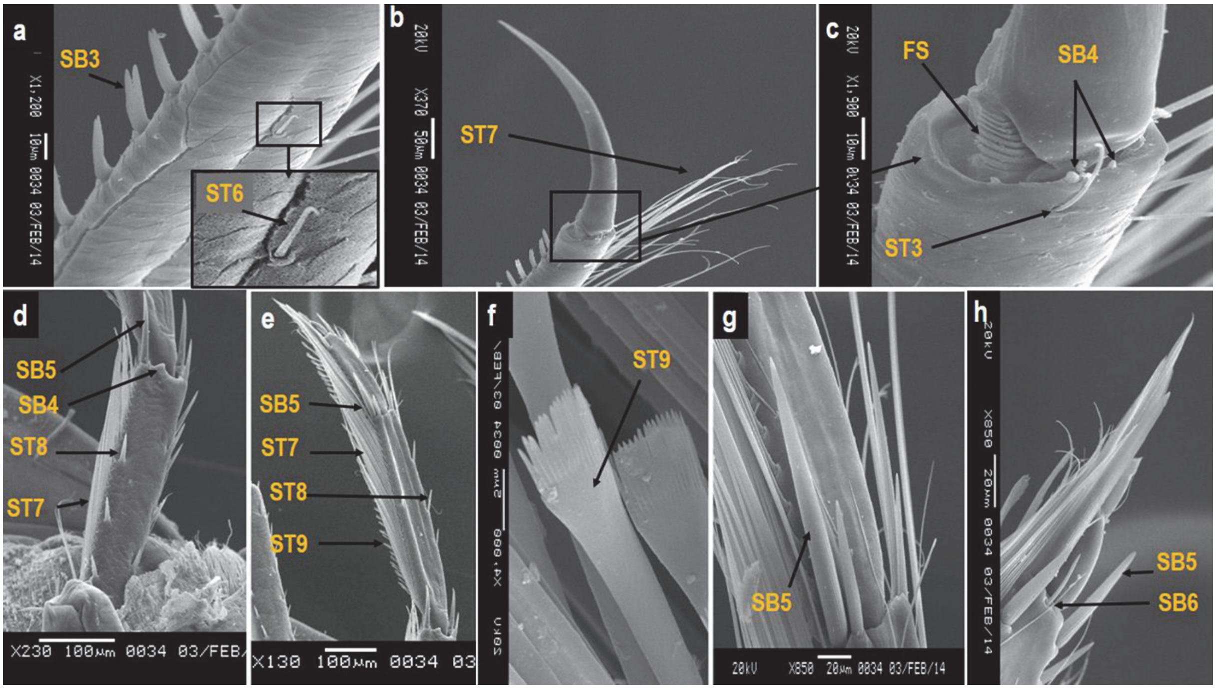

Fig. 3 *Micronecta haliploides*: a) Row of bifurcated sensilla basiconidea3 (SB3) and a single hook shaped sensilla trichoidea6 (ST6) with collar at its flexible socket is seen on the mid tarsus. b) Long, thin non socketed sensilla trichoidea7 (ST7) and folded structure on the base of the claw/ long digitalis. c) Enlarged view of the folded structure (FS) with its base possessing broad based stout tooth like sensilla basiconidea4 (SB4) and a hook shaped sensilla trichoidea3 (ST3) without collar on its flexible base. d) Hind femur with sensilla trichoidea8 (ST8) and long swimming hairs like sensilla trichoidea7 (ST7) aligned on the edges. Sensilla basiconidea4 (SB4) and sensilla basiconidea5 (SB5) on the apical portion of the hind femur. e) Hind tibia with sensilla trichoidea8 (ST8) and flat brush shaped sensilla trichoidea9 (ST9) and sensilla trichoidea7 (ST7). f) Enlarged view of sensilla trichoidea9 (ST9). g) The intersegmental region between the hind tibia and tarsus showing thick socketed sensilla basiconidea5 (SB5). h) Sensilla basiconidea5 (SB5) and sensilla basiconidea6 (SB6) at the base of the claw.

trichoidea13 (ST13) on a non-flexible socket (Fig. 5c). The curvature of these ST13, is away from the cuticle. Femur and tarsus of mid leg is also fully covered by ST13 but the intersegmental area between them shows bare undulating surface (Fig. 5d). The intersegmental area between tibia and tarsus of mid leg, is surrounded by relatively thick row of sensilla trichoidea14 (ST14) (Fig. 5e). Length of these sensilla ranged from 20- 60µm. These sensilla are arranged in a group of about 20- 25 sensilla aligned on non-flexible socketed base.

The basal portion of the hind femur shows presence of both sensilla ST11 and ST12 (Fig. 5f) that increases towards the apical part. The pattern of sensilla in the intersegmental area of hind femur and tibia is similar to the intersegmental region of the mid femur and tibia. Very few sensilla basiconidea (SB5) are seen in one or two places among the ST13 sensilla trichoidea covering the apical part of hind femur and basal part of hind tibia. In between these two, the undulating joint part is bare (Fig. 5g). Again, on the hind leg tarsus and tibia, sensilla ST14 are seen as in the intersegmental region between tibia and tarsus of mid leg (Fig. 5h). Hind tarsus is covered by non-flexible socketed sensilla trichoidea15 (ST15) (Fig. 5i). Unlike ST13, the curvature of these fine curved sensilla is towards the cuticle.

The sensory structures on the leg appendages of *M. haliploides* and *H. greeni* showed variation in size

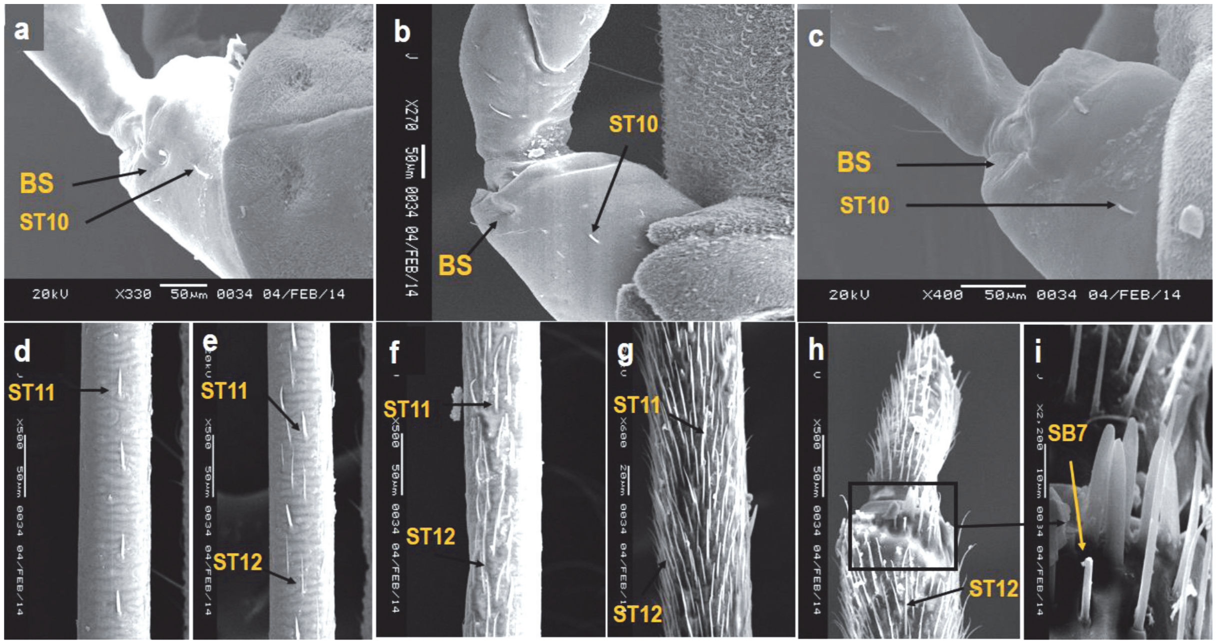

Fig. 4 *Hydrometra greeni* :a) Fore coxa, b) mid coxa and c) hind coxa, with a bell mouthed porous sensilla (BS) and a thorn like sensilla trichoidea10 (ST10). d) Base of the fore femur with thick sensilla trichoidea11 (ST11); e) Middle part of fore femur showing ST11 and a thin spine like rows of Sensilla trichoidea12 (ST12). f) Apical part of fore femur. g) Fore tibia covered by sensilla ST11 and ST12. h) The intersegmental area between fore tibia and tarsus is covered with long sensilla trichoidea12 (ST12). i) Enlarged view of the intersegmental portion showing relatively thick, spine like sensilla basiconidea7 (SB7) supported on a flexible socket on an undulating surface.

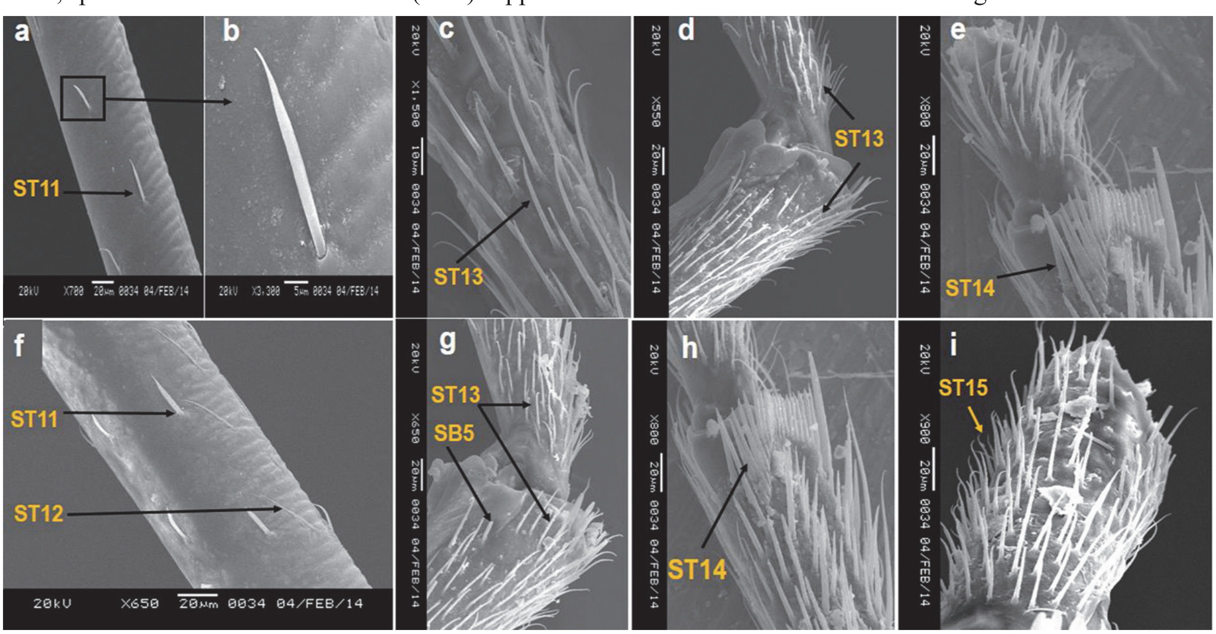

Fig. 5 *Hydrometra greeni*: a) Basal part of mid femur showing sensilla trichoidea ST11. b) Enlarged view of ST11 sensilla trichoidea. c) Apical part of mid femur with sensilla trichoidea13 (ST13. d) Sensilla (ST13) on mid-femur and tarsus and the undulating surface on the intersegmental area. e) Intersegmental area between mid-tibia and tarsus shows spine like sensilla trichoidea14 (ST14). f) Basal part of hind femur showing both ST11 and ST12. g) The intersegmental area between hind tibia and hind tarsus showing sensilla basiconidea5 (SB5) along with sensilla ST13 on the apical portion of femur. h) The intersegmental area between hind-tibia and hind tarsus is covered by sensilla trichoidea (ST14). i) Hind tarsus covered by sensilla trichoidea15 (ST15).

and shape among the two species and in different segments of their legs. Based on size, shape, pattern, their number and cuticular attachment (socketed or non-socketed), 26 types of sensilla were marked in these two species (Table 1). They are basically of 5 types as: sensilla trichoidea, sensilla basiconidea, sensilla placoidea, some porous circular sensilla and sensilla bell mouthed. Sensilla trichoidea (ST) are long, slender, hair-like structures with thin distal tip. Sensilla basiconidea (SB) are relatively shorter, stouter digitiforms with broad base and blunt distal tips. Both ST and SB are found buried inside depressions on the cuticle that can either be flexible socketed or non-flexible socketed and with collar or without collar. Sensilla placoidea (SP) are concave depressions. Sensilla bell mouthed is a funnel shaped, wavy cuticular fold with a pit (Nowiñska and Brożek, 2017).

Legs of *M. haliploides* are mainly comprised of sensilla trichoidea followed by sensilla basiconidea. More or less similar sensilla ST1 found on the coxa of this species were also reported in *Anisops sp.* (Gupta, 2008). Sensilla trichoidea are known to function as a mechanoreceptor (Nowiñska and Brożek, 2017). Sensilla placoidea (SP) on the dorsal side of the pala *M. haliploides* shows smooth surface. A study on antennomeres of adult females of *Cotesia sesamiae* and *Cotesia flavipes* reported sensilla placoidea as a sponge-like surface indicating that the structure is porous (Obonyo *et al.* 2011). Sensilla placoidea functions as an olfactoreceptor (Gao *et al*., 2007; Nowiñska and Brożek, 2017; Obonyo *et al.,* 2011).

The multi-porous circular sensilla (Cs) structures on the mid femur have a specific morphological structure. It is devoid of any hairy forms and each sensilla is found to be composed of numerous pores and slits. Each sensilla are about 10µm distant apart from each other. Multiporous sensilla are characterised as chemoreceptors and functions as olfactory sensilla (Nowiñska and Brożek, 2017). The folded structure on the base of the hind claw or the long digitalis of *M. haliploides* resembles the stridulatory file reported on the abdomen of *Micronecta burillu* (Bailey, 1983). Stridulation is well known among the Micronectidae (Reid *et al*., 2017; King, 1999). Lawson and Chu (1971) reported similar structures on the wings of Hemiptera-Homoptera bug, *Galgupha ovalis*.

In *M. haliploides,* sensilla ST3 (found on the ventral side of pala and base of the claw) is quite similar to the sensilla ST6 (found on the mid tarsus of *M. haliploides)* unlike the presence of a collar on the socket of ST6. Similar sensilla is also reported in *Amemboa cristata*, *Amemboa brevifasciata* and *Onychotrechus esakii* (Nowiñska and Brożek, 2017) as densely and uniformly distributed sensilla on the first and third antennomers whereas in the legs of *M. haliploides* it is noted singly and no other cuticular structure or sensilla was found surrounding it. Sensilla possessing a flexible socket are predicted to function as mechanoreceptors, and they receive stimuli by being touched, moved or deformed (Nowiñska and Brożek, 2017). The position of sensilla ST9 found on the tibia and tarsus of hind leg, in the midst of swimming hairs and their characteristic shape as flat brushes implies that these sensilla might function as mechanoreceptors for sensing the pressure or temperature of the water and may accomplish a mechanical role in either propelling or halting the insect in water current. Mechanoreceptors have the ability to perceive external stimuli during locomotion, oviposition and feeding (Nowiñska and Brożek, 2021).

H. greeni on the other hand shows different types and numbers of cuticular structures on its legs. The bell mouthed porous sensilla (BS) on its coxa is also reported as a new sensilla on the antennae of *Aquarius paludum,* another Gerromorpha bug belonging to the family Gerridae (Nowiñska and Brożek, 2017). The presence of such characteristic morphological structures on selective Gerromorpha bugs signifies their unique function that need further study. The gradual increase in the sensory structures from the base of femur to the apex of the tarsus of *H. greeni* is also reported in Gerridae, *Halobates germanus,* and *Aquarius elongates* (Perez-Goodwyn, 2009). Such structures provide support and liberty in locomotion on water surface. The densely spread cuticular structures provide hydrophobicity and distribute its body weight over

Table 1 List of sensory structures with morphplogy on the legs of *Micronecta haliploides and Hydrometra greeni*

Sl no	Structures	Morphology	Position on the specimen leg
1	Sensilla trichoidea1 (**ST1**)	Pliable, hair like, length less than 20µm	Fore, mid and hind coxae of *M. haliploides*
2	Sensilla trichoidea 2 (**ST2**)	Pointed, pliable, hair like, almost 50µm in length, embedded on a flexible socked with collar.	On Palar margin of *M. haliploides*
3	Sensilla trichoidea 3 (**ST3**)	Curved sensilla, with a hook like structure on its tip, embedded on flexible socket without collar.	Ventral side of pala and near the base of the mid leg claw of *M. haliploides*
4	Sensilla trichoidea 4 (**ST4**)	Pointed, pliable, hair like. More than 50µm in length, embedded on a flexible socked without collar alongside of ST3.	Ventral side of pala of *M. haliploides*
5	Sensilla trichoidea 5 (**ST5**)	Long slender sensilla with raised flexible socket.	Mid tibia of *M. haliploides*
6	Sensilla trichoidea 6 (**ST6**)	Small sensilla with curved tip embedded on a flexible sockets with collar.	Mid tarsus of *M. haliploides*
7	Sensilla trichoidea 7 (**ST7**)	Row of long, very thin hair like, pliable sensilla, embedded on non-flexible socket.	Mid tarsus, mid tibia and entire Hind leg of *M. haliploides*
8	Sensilla trichoidea 8 (**ST8**)	Broad based, relatively stout, pointed and non-flexible.	Hind femur of *M. haliploides*
9	Sensilla trichoidea 9 (**ST9**)	Paint brush shaped, flat.	Along with sensilla ST7 in *M. haliploides*
10	Sensilla trichoidea 10 (**ST10**)	Thorn like non flexible socketed.	Fore, mid and hind coxae of *H. greeni*
11	Sensilla trichoidea 11 (**ST11**)	Small, slender but thick, non-flexible socketed.	Fore femur of *H. greeni*
12	Sensilla trichoidea 12 (**ST12**)	Relatively thinner than ST11, spine like, non-flexible socketed.	Fore femur of *H. greeni*
13	Sensilla trichoidea 13 (**ST13**)	Array of pliable, thin smoothly curved, non-flexible socket.	Mid and hind femur of *H. greeni*
14	Sensilla trichoidea 14 (**ST14**)	It is a group of sensilla aligned at nonflexible socketed base, length of these sensilla ranged from 20- 60µm.	Intersegmental area of tibia and tarsus of mid and hind leg of *H. greeni*
15	Sensilla trichoidea 15 (**ST15**)	Array of pliable, thin smoothly curved, non-flexible socket. These are curved towards the cuticle.	Hind tarsus and tibia
16	Sensilla basiconidea1 (**SB1**)	Pointed tipped, straight, stout, 10µm in length, thick flexible base without collar.	Mid femur of *M. haliploides*
17	Sensilla basiconidea 2 (**SB2**)	Ribbed, stout, uniformly tapered tip with broad base and flexible socketed.	Mid tibia of *M. haliploides*
18	Sensilla basiconidea 3 (**SB3**)	Thick, bifurcated pointed tipped sensilla	Mid tarsus of *M. haliploides*
19	Sensilla basiconidea 4 (**SB4**)	Small thick, tooth-like.	Base of mid leg claw of *M. haliploides*
20	Sensilla basiconidea 5 (**SB5**)	Strong, thick, elongated, well-built flexible socket.	Base of mid leg claw and Intersegmental areas of mid and hind legs of *M. haliploides*

Sl no	Structures	Morphology	Position on the specimen leg
21	Sensilla basiconidea 6 (**SB6**)	Round, thin, relatively short digitiform, on well-built flexible socket.	Tip of the hind tarsus of *M. haliploides*
22	Sensilla basiconidea 7 (**SB7**)	Round, thin, relatively short digitiform, without collar.	Intersegmental area between fore-tibia and tarsus of *H. greeni*
23	Sensilla Placoidea (**SP**)	Concave depressions .	Dorsal side of pala of *M. haliploides*
24	Circular multi-porous sensilla (**CS**)	Consists of about 30 to 35 slits in a circular form.	Mid femur of *M. haliploides*
25	Folded cuticular structure (**FS**)	Multi-layered, resembles stridulatory organs.	Base of mid leg claw of *M. haliploides*
26	Bell mouthed sensilla (**BS**)	Conical shaped, porous sensilla, pit enclosed inside an undulating cuticular cone.	Fore, mid and hind coxae of *H. greeni*

a large area of the surface film for support on the water surface (Andersen, 1989).

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Detection of Zika virus in *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae) in India - First report

M. S. Sasi[1*], R. Rajendran[2], V. Meenakshy[1], T. Suresh[3], R. Heera Pillai[4], T. Dilip Kumar[5], Athira Sugathan[6] and K. Regu[2]

[1]Directorate of Health Services, Govt. of Kerala, Thiruvananthapuram 695035, Kerala, India; [2]National Centre for Disease Control, Kozhikode 673003, Kerala, India; [3]District Vector Borne Disease Control Office, Health Services Department, Thiruvananthapuram 695035, Kerala, India; [4]Laboratory Medicine and Molecular Diagnostic, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695014, Kerala, India; [5]Regional Office of Health and Family welfare, Thiruvananthapuram 695035, Kerala; [6]State IDSP cell, Directorate of Health Services, Thiruvananthapuram 695035, Kerala, India. Email: adentomology@gmail.com; rajendran061@gmail.com

ABSTRACT: Zika virus (ZIKV) a mosquito-borne, causing acute febrile illness associated with rash, arthralgia and conjunctivitis in the patient, was reported from Thiruvananthapuram, Kerala, as an outbreak with 83 cases. Entomological surveillance revealed the presence of aedine mosquitoes *viz., Aedes aegypti* (Linnaeus, 1762), *Ae. albopictus* (Skuse, 1894) and *Ae. vittatus* (Bigot, 1861) and non-aedine mosquitoes *viz., Anopheles stephensi* Liston,1901, *Mansonia uniformis* (Theobald, 1901), *Culex tritaeniorhynchus* Giles, 1901 and *Cx. gelidus* Theobald,1901. *Aedes* (*Ae. aegypti, Ae. vittatus and Ae. Albopictus*) mosquito larvae were high in the Zika affected areas. Moreover ZIKV was detected in *An. stephensi* mosquitoes collected from Parassala, Thiruvananthapuram (the native place of the first ZIKV confirmed case in the present outbreak in Kerala). Molecular diagnostics of *Ae. Aegypti, Ae. vittatu* and *An. stephensi* mosquitoes revealed that the species were loaded with ZIKV. Significantly this is the first ever report of ZIKV detecting in *An. stephensi* in the world. *Aedes* adults (male and female) and *An. stephensi* emerged from fourth instar larvae and pupae were found to have ZIKV, indicating transovarial transmission of the virus.

KEY WORDS: Flavivirus, entomological surveillance, vector incrimination, RTPCR, Kerala

INTRODUCTION

Zika virus (ZIKV) is a mosquito-borne *flavivirus* closely related to human pathogenic viruses such as dengue, chikungunya, yellow fever, Japanese encephalitis and West Nile viruses, and is a public health concern all over the world. ZIKV was first detected in *Aedes* (*Stegomyia*) *africanus* (Theobald, 1901) mosquitoes from Zika forest of Uganda (Dick *et al*., 1952). ZIKV originally circulated in enzootic cycles between forest dwelling canopy-feeder mosquitoes and non-human primates (Weinbren and Williams, 1958). The virus caused outbreaks in different Pacific regions during the period from 2007 to 2015 and began extending throughout the Americas in 2015 (Musso and Gubler, 2016). The ability of ZIKV to cause congenital defects in newborn, as evidenced by the

* Author for correspondence

microcephaly epidemic in Brazil, has been an unprecedented characteristic in a mosquito-borne viral infection (de Oliveira *et al*., 2017). Although transmission of ZIKV has declined in the Americas, Zika fever outbreaks continue to occur in South East Asia including India (Grubaugh *et al.,* 2019). It was first reported in Asia during 1966. India reported ZIKV infections from 2016 onwards from Gujarat, Tamil Nadu, Rajasthan, Madhya Pradesh and Kerala (Sasi *et al*., 2021). The first confirmed ZIKV case from Uttar Pradesh was detected in Kanpur on 24th October 2021 as confirmed by National Institute of Virology, Pune. In Rajasthan, ZIKV was detected in *Aedes aegypti* (Linnaeus, 1762) mosquitoes collected from Jaipur (Singh *et al*., 2019). As of latest, Sasi *et al.* (2021) reported ZIKV in *Ae. aegypti, Ae. albopictus* (Skuse, 1894) and *Ae. vittatus* (Bigot, 1861) from Thiruvananthapuram, Kerala.

The virus caused a perceptible Pan-American epidemic after its first advent in Brazil in 2015 (Brasil *et al*., 2016). Though the virus can scarcely be transmitted between humans, transmission by mosquito bite is considered to be the most common way of virus dissemination in disease outbreak zones (Musso and Gubler, 2016). ZIKV was first reported in East Africa in 1947 and expanded from lineal enzootic cycle in Africa to Asia. At the beginning of the 21st century, the virus expanded into the South Pacific and Americas, triggering a pandemic that led to 87 countries or territories reporting active ZIKV transmission by 2021. The first documentation of inter-human urban transmission of ZIKV through *Aedes* (*Stegomyia*) *aegypti* came from Malaysia in 1966. *Aedes aegypti* is a known vector of dengue, chikungunya and yellow fever (Rajendran *et al*., 2021). Many investigators attempted to detect the virus during outbreaks, but the virus could be detected in few instances (Akoua-Koffi *et al*., 2001). Chouin-Carneiro *et al*. (2016) established *Ae. aegypti* as the vector in spreading the virus in laboratory assays. Natural infections of ZIKV have been detected in several mosquito species during outbreaks in Africa. However, viral detection in mosquitoes in urban outbreak areas has been scarce to non-existent (Musso and Gubler, 2016). Vector incrimination study is crucial in ZIKV transmission dynamics (Gutierrez- Bugallo *et al.,* 2019).

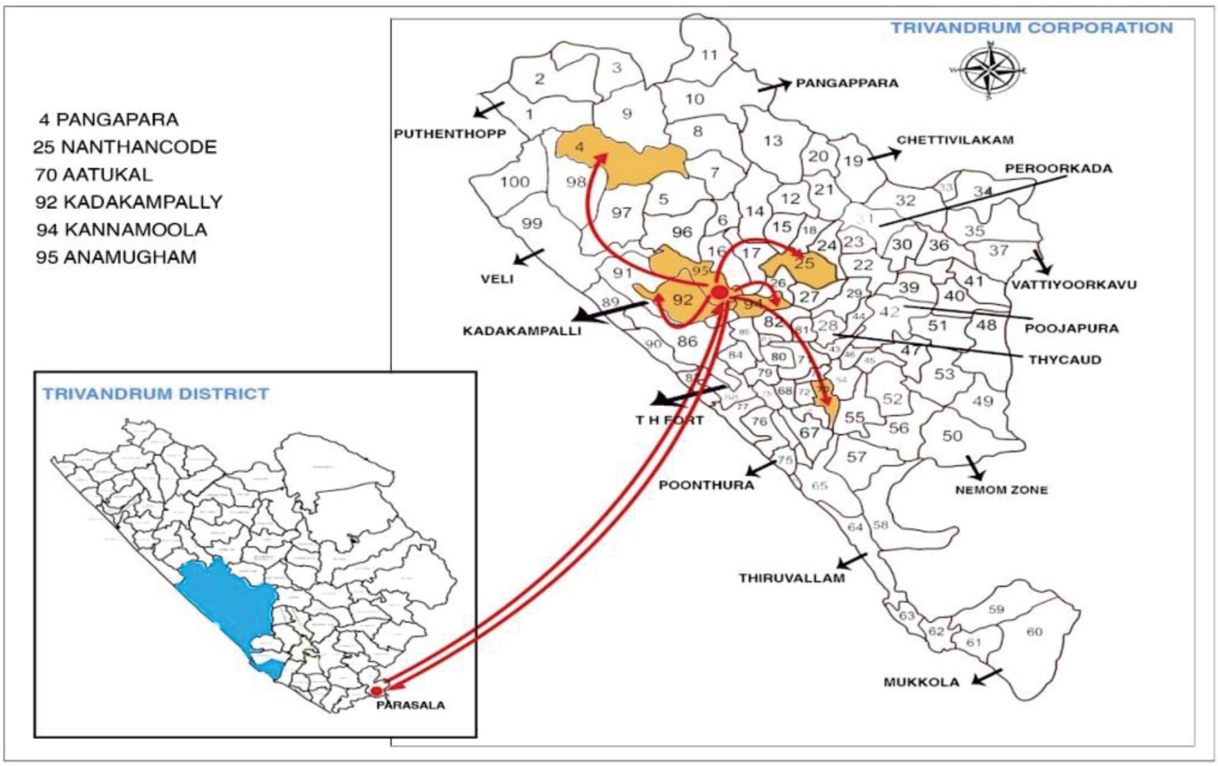
The first confirmed ZIKV case of Kerala was reported from Thiruvananthapuram on 8th July 2021. Since then, 83 ZIKV positive cases have been reported from the state. Study on vector prevalence in the outbreak area and their possible role in transmitting the virus forms an integral part in the assessment of the magnitude and severity of disease transmission in a locality. Hence, extended vector surveillance was carried out in the disease affected areas of Thiruvananthapuram district to ascertain the potential role of vector-pathogen relationship and dynamism of disease transmission.

MATERIALS AND METHODS

Study area: The first confirmed case of ZIKV was reported in a pregnant woman admitted in a hospital located in Anamugham (Ward No.95) of Thiruvananthapuram Municipal Corporation (TMC), Kerala. In the initial months, she stayed in Parassala (her native place), and during the final months of her gestation, she stayed in Nandancode (Ward No. 25), TMC (Map 1). Subsequent ZIKV cases had apparently contracted the infection from the limits of TMC. Hence, a study involving vector surveillance and vector incrimination was planned and carried focusing TMC area.

Entomological surveillance: During the recent ZIKV outbreak, detailed vector surveillance was carried out (from 8th to 27th July 2021) in 10 wards in TMC (Anamugham (Ward No. 95), Nandancode (Ward No. 25), Parassala, Palkulangara (Ward No.85), Kadakampally (Ward No.92), Kunnukuzhi (Ward No.26), Kannanthura (Ward No.84), Thycaud (Ward No.28), Pattom (Ward No.17), Balaramapuram (Ward No.19) and Anayara (an area within Ward No.86), and 80% of them were micro containment wards for infection. All accessible water holding containers /habitats in and around houses/other building premises were checked for the presence of mosquito larvae/pupae (Rajendran *et al*., 2021) in the Zika outbreak areas. Larvae/pupae collected from each of the containers/sources were kept in separate vials labeled with date of collection, locality, house

MAP 1. ROUTE MAP OF FIRST ZIKA CONFIRMED CASE IN THIRUVANANTHAPURAM, KERALA



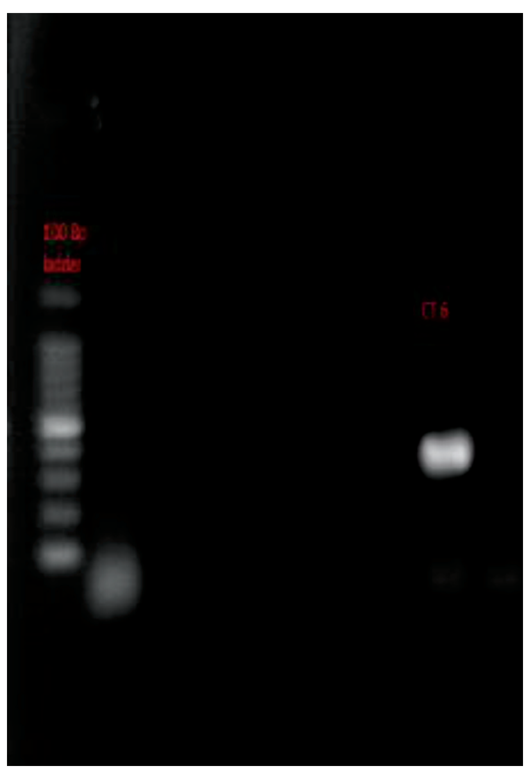


Fig.1 ZIKA virus positive gel image

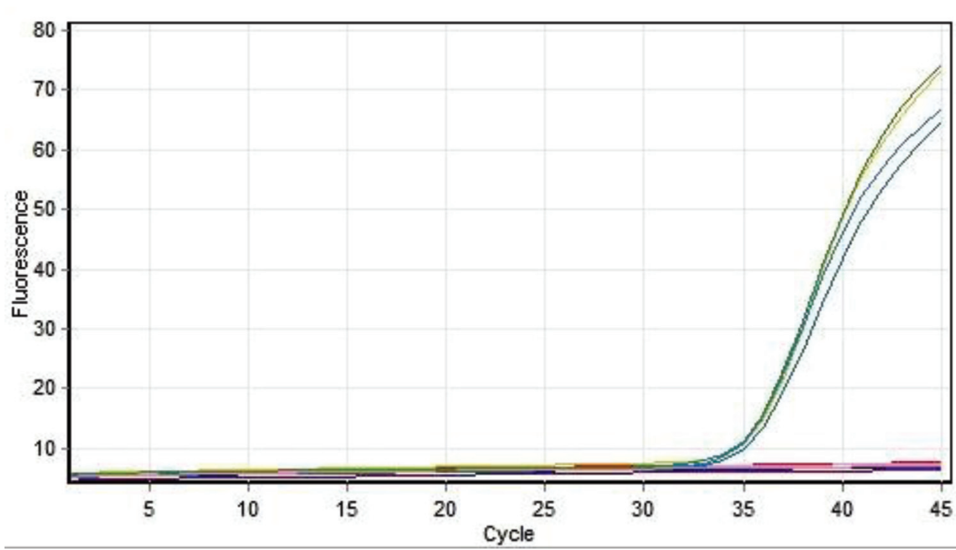


Fig. 2 Raw Data for Cycling A. Green

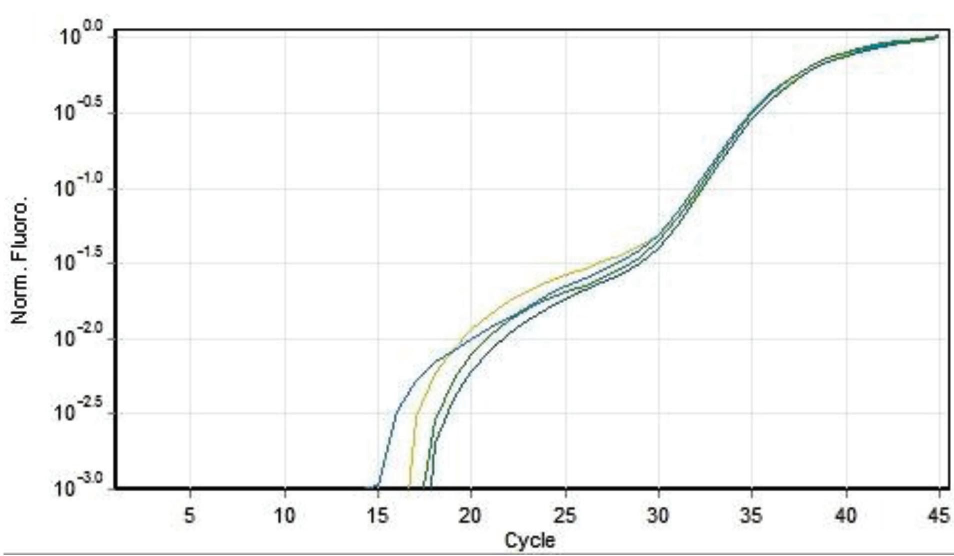


Fig. 3 Quantitation data for Cycling A.Green

number and breeding source (container type/ habitat), and reared in the State Entomology Unit laboratory attached to the Directorate of Health Services (DHS), Thiruvananthapuram, Kerala. Collected larvae/pupae were reared in jars filled with 150 ml fresh water, covering with fine mosquito net. The field collected adult mosquitoes as well as the adults emerged from the larvae/pupae were identified using standard key (Rueda, 2004; WHO, 2020). The identified male and female mosquitoes were kept in separate pools for virus detection as per standard procedure.

Detection of Zika virus: Samples of mosquitoes from different pools labeled with identity details were analysed for virus detection using RT-PCR/ virus isolation protocols at Rajiv Gandhi Centre for Biotechnology (RGBC), Thiruvananthapuram, Kerala.

Viral Nucleic Acid (RNA) isolation: The specimens stored in 70% ethanol were put into a fresh sterile tubes and added 100µl 1x PBS to each tube. The specimen was washed with 1X PBS by tapping the tube gently and 1X PBS was decanted. This procedure was done to wash off the ethanol thoroughly, as traces of ethanol will inhibit PCR. Cryogenic grinding, otherwise known as freeze grinding, technique was used for grinding tissue samples to extract nucleic acid. The specimens were cooled below -80^{0}C by adding liquid nitrogen. The specimens were subjected to cryogenic grinding using liquid nitrogen in mortar and pestle under aseptic conditions. 200µl 1X PBS (lysis buffer) was added to the mortar with the powdered specimen. The powder was dissolved completely. The solution was then transferred to 1.5ml micro centrifuge tube and was centrifuged at 13,000 rpm for 5 minutes at room temperature. The supernatant was discarded. 200µl 1X PBS was again added to the pellet and was vortexes for 1 minute until the pellet was completely dissolved. Viral nucleic acid (RNA) isolation from the supernatant was carried out using QIAGEN s QIAamp Viral RNA Extraction Kit®(QIAGEN, Germany), following the manufacturer's protocol.

Identification of Zika virus diversity in vector mosquito: A direct PCR amplification reaction was achieved for identification of zika virus using remoted viral RNA immediately with unique primers. The isolated viral RNA was directly used as the template. The PCR merchandise was then electrophoresed on 1.5 per cent agarose gel in 1x TAE buffer. 0.5 µl of forward and reverse primers along with TaKaRa's prime script one step Rt-PCR master blend (TaKaRa, Japan) including 12. 5 µl of 2x one step RT buffer, 0.5 µl of taq polymerase (5 units/µl), 0. 5 µl of 5x different transcriptase enzyme and 3.0 µl of RNAse unfastened dH2O. A total reaction volume of 25µL was subjected to PCR for 40 cycles, with an initial cDNA synthesis step at 42^{0} C for 5 min., initial denaturation at 94^{0} C for 10 secs, denaturation at 94^{0} C for 30 secs, annealing at 55^{0} C for 60 sec., and extension at 72^{0} C for 60 sec., and a final extension at 72^{0} C for 60 sec. The products were analyzed as bands on a 1.5% Agarose gel in 1X TAE buffer at 250 Bp. Positive bands obtained were extracted from the gel and purified. The purified product was then subjected for cycling sequencing followed by Sanger Sequencing. The sequence obtained was analysed using NCBI BLAST. RT-PCR was performed using RealStar® Zika Virus RT-PCR Kit 1.0, Altona Diagnostics, GmBH, Germany according to manufacturer's protocol.

RESULTS AND DISCUSSION

The first case of ZIKV in Kerala was reported from Nandancode (Ward No.25) of TMC area. On suspected symptoms, the serum sample of the patient was subjected to laboratory tests and confirmed for Zika positive by National Institute of Virology, Pune. The investigation team visited the residential area of the first Zika confirmed case on 8th July 2021 to trace the primary source of the infection. The duration of the stay of the first Zika confirmed case in the assigned area, incubation period of the disease and vector proliferation magnitude suggest that the first confirmed case might have interacted with many people in Nandancode, where the patient resided during the onset of illness. There had been a history of as many as 14 staff of the mentioned hospital presented with fever symptoms as early as in mid of May 2021, and 19 samples of staff subjected to test for measles, rubella, dengue and chikungunya

found negative at NIV, Pune, but test for Zika was not done at that time due to lack precedence. However, after the confirmation of first ZIKV case, the aforesaid 19 archived samples were retrieved and tested for Zika, of which 14 were found positive.

Extensive vector surveillance carried out in Zika affected areas revealed the presence of aedine mosquitoes *viz.*, *Ae. aegypti, Ae. albopictus* (Skuse, 1894) and *Ae. vittatus* (Bigot, 1861) and non-aedine mosquitoes *viz*., *Anopheles stephensi* Liston, 1901, *Mansonia uniformis* (Theobald, 1901), *Culex tritaeniorhynchus* Giles, 1901, *Cx. gelidus* Theobald, 1901. A total of 137 males and 108 females were collected from the wards. Further the State Entomology team surveyed in and around the house of the first Zika confirmed case in Parassala, but could not detect any mosquito breeding habitats. However, the team could collect five *Anopheles* fourth instar larvae and four pupae from a deserted fish (cement) tank in the terrace of a neighbouring building. On emergence (only three adults), the mosquitoes were identified as *An. stephensi,* a known vector of malaria in Kerala and elsewhere.

All the samples (adult mosquitoes that emerged from fourth instar larvae collected) were screened primarily using Real Time PCR for the detection of Zika Virus. Fig.1 shows ZIKA virus positive gel image. The four sigmoid graphs in the raw data (obtained in real time PCR) represents positive control (Fig. 2); while the four sigmoid graphs represents PCR positive samples isolated from the adults of *Ae. aegypti, Ae. vittatus* and *An. stephensi* (Fig. 3). Based on the diagnostics, ZIKV was detected in the *Ae. vittatus* mosquitoes (7 males) from Nandancode and in *Ae. aegypti* (3 males and 3 females) from Anamugham. Three female *An. stephensi* mosquitoes obtained from rearing were found positive for Zika virus (Figs. 1, 2, 3). Detection of ZIKA virus in these three species, (reared from fourth instar larvae from Zika affected localities), indicates the transovarial transmission of the virus.

ZIKV detected in *Ae. aegypti* mosquitoes collected from Zika outbreak areas of Rajasthan in 2019 (Singh *et al*., 2019) was the first report of the detection of ZIKV in *Ae. aegypti* mosquitoes in India. Sasi *et al.* (2021) reported detection of ZIKV in the *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* from Thiruvananthapuram, Kerala. *Anopheline* mosquitoes all over the world indicate that only *An. africanus*, *An. coustani* Laveran, 1900 and *An. gambiae* Giles, 1902 are incriminated vectors of ZIKV. As of the case, this is the first ever global report of *An. stephensi* for ZIKV detection and transovarial transmission. During the present investigation, ZIKV could not be detected in *Mansonia uniformis* (Theobald, 1901), *Culex tritaeniorhynchus* Giles, 1901, *Cx. gelidus* Theobald, 1901 mosquitoes collected from Zika affected areas. The detection of ZIKV in the reared *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* confirms the transovarial transmission of the virus as reported by Lai *et al*. (2020).

Since first ZIKV was detected in *Ae. africanus* mosquitoes collected from Zika forest, Uganda in 1948 (Dick *et al*., 1952), its positivity has been found in 20 species of *Aedes* mosquitoes, one species each of *Mansonia* and *Culex* and three species of *Anopheles* mosquitoes so far from different Zika affected areas of the world. In India, ZIKV has been detected in *Ae. aegypti, Ae. albopictus and Ae. vittatus* mosquitoes. As per the current report, there are 31 wild-caught mosquito species infected with ZIKV worldwide. These mosquitoes belong to *Aedes* (22 species), *Culex* (4 species), *Anopheles* and *Eretmapodites* (2 species each) and *Mansonia* (1 species). Among the *Aedes* mosquitoes, ZIKV could be detected from nine species of mosquitoes belonging to the subgenus *Stegomyia*. This suggests that *Aedes* genus, especially *Stegomyia* subgenus is the most significant taxon involved in ZIKV transmission. So far, ZIKV could be detected in 21 species of mosquitoes collected from sylvatic settings. However only six species (*Ae. aegypti*, *Ae. albopictus*, *Ae. vexans* Meigen, 1830, *Cx. quinquefasciatus* (Say), *Cx. coronator* (Dyar and Knab) and *Cx. tarsalis* Coquillett, 1896 have been identified as ZIKV vectors in urban settings (Smartt *et al*., 2017; Elizondo-Quiroga *et al*., 2018).

The present study reveals that in addition to the conventional *Aedes* mosquitoes, Zika virus also has

An. stephensi as a potent vector. Significantly this is the first ever report of ZIKV detecting in *An. stephensi* in the world. *Aedes* adults (male and female) and *An. stephensi* emerged from fourth instar larvae and pupae were found to have ZIKV, indicating transovarial transmission of the virus. This obviously calls for a change in vector control strategy, especially in the areas where these mosquitoes are abundant. Most of the districts of Kerala, especially coastal areas, are conducive for profuse breeding of *Ae. aegypti*, *Ae. albopictus*, *Ae. vittatus* and *An. stephensi*, the vectors of ZIKV in the recent outbreak. There is a need for continuation of human, veterinary, entomological and environmental surveillance to ascertain the incidence and geographical distribution of ZIKV, host and vector diversity, virus strain and its transmission potential. Effective surveillance and appropriate vector control strategy are a must to avert future outbreaks.

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Report of *Lissachatina fulica* (Bowdich, 1822) (Stylommatophora: Achatinidae) in rubber plantations of Western Ghats, Kerala

Keerthy Vijayan[1*], R. Suganthasakthivel[2], T.V. Sajeev[3] and Fred Naggs[4]

[1]*School of Agriculture and Horticulture, Kalasalingam Academy of Research and Education, Krishnankoil 626 128, Tamil Nadu, India;*
[2]*71, South Agraharam, Watrap 626 132, Tamil Nadu, India;*
[3] *Forest Entomology Department, Kerala Forest Research Institute, Peechi 680 653, Kerala, India;*
[4]*Department of Life Sciences, The Natural History Museum, London, United Kingdom.*
Email: kimmumolath@gmail.com

ABSTRACT: The giant African snail *Lissachatina fulica* (Bowdich, 1822) is reported as a pest in rubber plantations adjoining forest fringes in the Western Ghats region of Kerala. The snail was causing damage to rubber (*Hevea brasiliensis)* and nutmeg (*Myristica fragrans)* trees, by feeding on rubber latex and nutmeg twigs and leaves. *L. fulica* infestation on *M. fragrans* is a new record. The snail infestation in rubber plantations is the first report from the Western Ghats region in Kerala.

KEYWORDS: African snail, rubber, nutmeg, Western Ghats, biological invasion

African snail *Lissachatina fulica* (Bowdich, 1822) is one of the largest gastropod molluscs and is considered to be one of the world's worst 100 invasive species (Lowe *et al.,* 2000). Native to coastal East Africa, it has been spreading to new areas since the early1800s (Raut and Barker, 2002). Its success as an invasive species can be attributed to their polyphagous habits, feeding on more than 500 species of plants (Lowe *et al.,* 2000)), adaptability, active dispersion and fecundity (Mead, 1979). The snail is a protandrous simultaneous hermaphroditic (Tomiyama, 1996). It acts as an intermediate host of the rat lung worm *Angiostrongylus cantonensis* causing eosinophilic meningitis in humans, especially children. When untreated, the disease can be fatal (Lv *et al.,* 2011). The snail thrives in warm and humid tropical climates and is active within a temperature range of 9 to 29° C, primarily nocturnal, it is also active throughout the day in wet conditions. They aestivate in extended dry conditions, when they may bury into the soil (Raut and Barker, 2002). Other than Antarctica, *L. fulica* have been recorded in all continents and are highly invasive in at least 52 countries (Global Invasive Species Database, 2019). The first recorded introduction into India was in 1847 into a garden in Calcutta (Naggs, 1997). The snail reached south India during the British period in My Lady Garden in Madras and later spread rapidly into many localities of Tamil Nadu (Raut and Ghose, 1984), reached Kerala during the 1950s. The first invasion in the 1960s as a pest was limited but a second wave of invasion or population explosions was observed during the 1970s. The present wave of invasion or population explosions started in 2005 and *L. fulica* is persisting

* Author for correspondence

as an agriculture pest in home gardens and in human settlements. Field surveys conducted in the states of Kerala and Tamil Nadu during the years 2016 to 2018, observed the presence of giant African snails (Keerthy *et al*., 2019).

During 2013 to 2019 surveys conducted revealed infestation of *L. fulica* in 269 localities in 13 out of 14 districts in Kerala (Table 1). During October 2019 and June 2020, *L. fulica* was reported feeding on rubber (*Hevea brasiliensis)* and on nutmeg trees (*Myristica fragrans)* in the rubber plantations belonging to the Plantation Corporation of Kerala. The locality is in one of 5 forest ranges in the Athirappilly forest range of the Vazhachal forest division in Western Ghats region of Kerala that adjoins moist deciduous forest. The coordinates of the locality were 76° 31' 22.537" East longitude and 10° 15' 51.948" North latitude at an elevation of 104 meters above mean sea level. The area from which the snails were reported is thick in vegetation with rubber, nutmeg and passion fruit *Passiflora* sp. Rubber and passion fruit trees have been recorded as economically important plants affected by the *L. fulica* (Raut and Barker, 2002). However, out of the hundreds of plant species affected by the snail so far (CABI, 2021), the nutmeg tree *M. fragrans* being damaged by *L. fulica* is reported here for the first time. Nutmeg is an economically important spice crop in Kerala and the potential for *L. fulica* to be a pest of nutmug is a matter of concern.

Table 1. Distribution of *Lissachatina fulica* in Kerala

District	*No.
Kasaragode	6
Kannur	16
Waynad	1
Kozhikode	10
Malappuram	6
Palakkad	61
Thrissur	11
Ernakulam	38
Alappuzha	15
Kottayam	1
Pathanamthitta	15
Kollam	18
Thiruvananthapuram	71
TOTAL	269

*No. of infested localities

Following repeated floods in Kerala in 2018 and 2019, *L. fulica* have appeared in new areas and at much higher density in some locations. In Kasaragode and Kollam districts, the snails were found to be in huge numbers and feeding on latex secreted from rubber trees causing damage. Routine transport of a range of material across plantations provides an unintended mode of transfer of *L. fulica* between plantations. In presenting favourable conditions, protracted rainfall increases both the incidence of dispersal and rapid population growth. The recent massive floods in Kerala will also have increased the opportunity for rapid and wide-ranging dispersal by flood waters. For example, the Chalakkudy River in flood could have transported *L. fulica* to forests in the Vazhachal forest division.

L. fulica was introduced into Sri Lanka in about 1900 (Green, 1911; Naggs, 1997) and was recorded as ‘attacking rubber trees’ in large numbers in the 1940s. The initial population explosions in Sri Lanka subsequently crashed to lower densities (Cotton, 1940). Nevertheless, *L. fulica* persists, often in large numbers, throughout Sri Lanka from coastal margins to the Central Highlands at over 1,000 m; it occurs in all climate zones and all surveyed habitat types (Naggs *et al*., 2003; Naggs and Raheem, 2005). In south America, *L. fulica* has become established in highly transformed urban areas adjacent to Paranese rainforest (Gregoric *et al.,* 2011) and in Amazonia (Goldyn *et al.,* 2017). In India, *L. fulica* has mainly been reported as a pest in mulberry gardens (Narendrakumar *et al.,* 2011), banana plantations (Padmanaban *et al.,* 2000), vanilla plantations (Vanitha *et al*., 2011) and agriculture gardens (Sridhar *et al*., 2012). Infestations in rubber plantations and the consumption of rubber seedlings have been reported from many places across the globe (Raut and Barker, 2002).

This is the first report of *L. fulica* infesting rubber plantations in Kerala and feeding on rubber latex, and nutmeg twigs and leaves, from plantation forests of the Western Ghats region in Kerala. Two other native molluscan species *Mariaella dussumieri* and *Crypotazona bistrialis* are limited pests to rubber in India (http://www.celkau.in/Crops/Plantation Crops/Rubber/pests.aspx).

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Oxyrachis tarandus Fab. (Homoptera: Membracidae) on rose apple (*Syzygium aqueum*)

Medammal Zubair[1] and Keethadath Arshad[2*]

[1]*Department of Zoology, University of Calicut, Malappuram 673635, Kerala, India;*
[2]*Department of Zoology, PSMO College, Tirurangadi, Malappuram 676306, Kerala, India.*
Email: akeethadath@gmail.com

ABSTRACT: *Oxyrachis tarandus* Fab. (Homoptera: Membracidae), commonly known as cow horn bug or treehopper was found heavily infested on rose apple (*Syzygium aqueum* (Burm.f.) Alston, Myrtaceae). Infestation caused wilting, defoliation and structural abnormalities of fruits in *S. aqueum* and was found in 81 patches within a tree, which is further divided into peduncle, PD (48 patches), young terminal branches, YTB (20), older twig, OT (13), main bark, MB (0) and leaf, L (0). Infestation of shoot length ranged from 3 to 25 cm comprising a surface area of 5.47 to 25.47 cm^2. Population density of cow horn bug was significantly higher in PD compared to YTB and OT and peak infestation was noted during last week of March and first week of April. Prominent mutualism between *O. tarandus* and ant *Oecophylla smaragdina* was noted with strong positive correlation.

KEY WORDS: Cow horn bug, new host, population density, ants, mutualism

Oxyrachis tarandus Fab (Homoptera: Membracidae), commonly known as cow horn bug or treehopper, with two characteristic lateral and a median horn of the pronotum, is a phytophagous insect, where nymphs and adults feed on tender shoots. The regular dark brown-to-black adults measure approximately 7 mm in length. They hop about when disturbed and this habit has earned them the popular name "tree hoppers" (Ananthasubramanian, 1996; Ranga rao and Shanower, 1999; Nettimi and Iyer, 2015; Prabakaran *et al.,* 2017). They exhibit diversity in behavioral and life history traits including maternal care (subsociality), ant mutualism, host-plant specialization and plant-borne vibrational communication (Wood, 1993; Cocroft, 1996, 2001). It is considered as the minor pest as they do not appear regularly and thus a sporadic pest. It is considered as the minor pest as they do not appear regularly and thus a sporadic pest. However, it may be getting the status of major insect pest in near future due to intensive cropping, higher dosages of fertilizers and variation in microclimate (Ranga rao and Shanower, 1999; Garg, 2015; Rahmathulla *et al.,* 2015).

The oviposition site of *O. tarandus* is on young shoots, petioles or leaf midrib in a V-shaped slit. Eggs dispersed in clusters are being protected inside plant tissue covered by a white secretion and defended by female members. Presence of mutulistic ants also governs further protection. They utilize immature, often differentiating tissues of host plants and their phenology is synchronized with

* Author for correspondence

growth season of the host plant. Within few weeks after egg laying, hatching takes place. Aggregations of nymphs become largest on most vigorous plant modules thus, feeding gregariously on the sap of the shoot. Nymphs pass through five developmental stages to complete the life cycle within 2 to 2.5 months under optimum conditions. *O. tarandus* is distributed over the host plant in patches on young modules. Its abundance is low over a landscape, but may become numerous locally, which is meant for a stable population dynamics at the landscape scale and unpredictable locally. Formation of corky calluses, wilting and reduced plant vigor are the symptoms of heavy infestation. They undergo diapause in adult stage during environmental stress (Borror *et al.*, 1992; Ranga rao and Shanower, 1999; Price and Carr, 2000). The association observed between treehoppers and attendant ants (Hymenoptera: Formicidae) is one of the most familiar mutualisms between animal species, which is recognized as a common and important ecological interaction (Buckley, 1987; Stachowicz, 2001). Attendant ants are benefitted through a sugary waste excretion called honeydew produced by bug. Bugs in turn are benefitted through the protection governed by ants from its predators and parasites. Rose apple (*Syzygium* spp) is a common fruit plant in the home gardens and is grown commercially as the fruits are of high demand in the market for its delicious taste.

The present study was motivated by observation of huge number of *O. tarandus* and associated ants on rose apple, *S. aqueum* (Burm.f.) Alston (Myrtaceae), in Kannur district of Kerala, India. Current observations are significant, as rose apple (*Syzygium* spp.) has not been reported as the potential host plant for *O. tarandus* so far and its host association and ecology are discussed. Three different species of rose apple, *Syzygium jambos* (L.) Alston*, S. samarangense* (Blume) Merr. & L.M.Perry and *S. aqueum* were observed for the infestation of *O. tarandus* during the present study. A total of 30 trees (10 each from a species) were selected at random sites of Kannur district (11.9709° N, 75.6208° E) were examined during February to March 2020 and repeated during 2021. Observed trees were having a height of 5 to 8 m. Each tree was divided into regions of one meter each from bottom to top (A, B, C… etc.) to analyze the latitudinal distribution of cow bug over a single tree. Within each region infestation was examined under five sub areas namely, young terminal branches (YTB, recognized by green fleshy stem with a diameter less than 1 cm), older twig (OT, recognized by brown, scaly thick stem, with diameter more than 1 cm), main bark (MB, main strong axial stem, varied thickness from 25cm (base) to 5cm (terminal), peduncle (PD) and leaf (L). Measurements were taken to find the length of each infestation patches. Number of adults and nymphs in each patch was recorded (Nettimi and Iyer, 2015). Population density of each patch was calculated as number of insects per unit surface area. Statistical analyses were performed using standard statistical software, Graphpad Instat™ (GraphPad Software, Inc., La Jolla, CA; 1990-1993 Graphpad Software. V2 00, Uchitel, UC Irvine 921687S) and the data were expressed as Mean ± SD. Student's t-test (one tail) was performed to analyse any significant difference between two groups. Correlation regression analysis were performed to analyse the mutualism between *O. tarandus* and ants. Photographs were taken using Canon EOS 70D Digital SLR Camera with 18-135mm STM Lens and EF 75-300mm f/4-5.6 III Telephoto Zoom Lens.

Out of the three plant species observed, only *S.aqueum* was found infested with *O. tarandus.* A well established latitudinal variation and effective utilization of available resources are seen over the tree. Initially it was infested on region B (2nd from bottom) then spread over upper regions one by one on increase in population size as reproduction is going on. Lower most region A was left free throughout the observation period; it could be due to lack of young branches. Upper most region was also left non-infested (Fig. 1h) and authors suggests this as a behaviour most probably to avoid direct sunlight. Average surface area of prominent patches, PD, YTB and OT was 6.9 ± 3.1, 21.6 ± 4.0 and 112.7 ± 48.3 cm^2 respectively. A total of 81 patches of cow bug infestation were observed during heavy infestation (last week of March). Lower and higher temperature noted during the

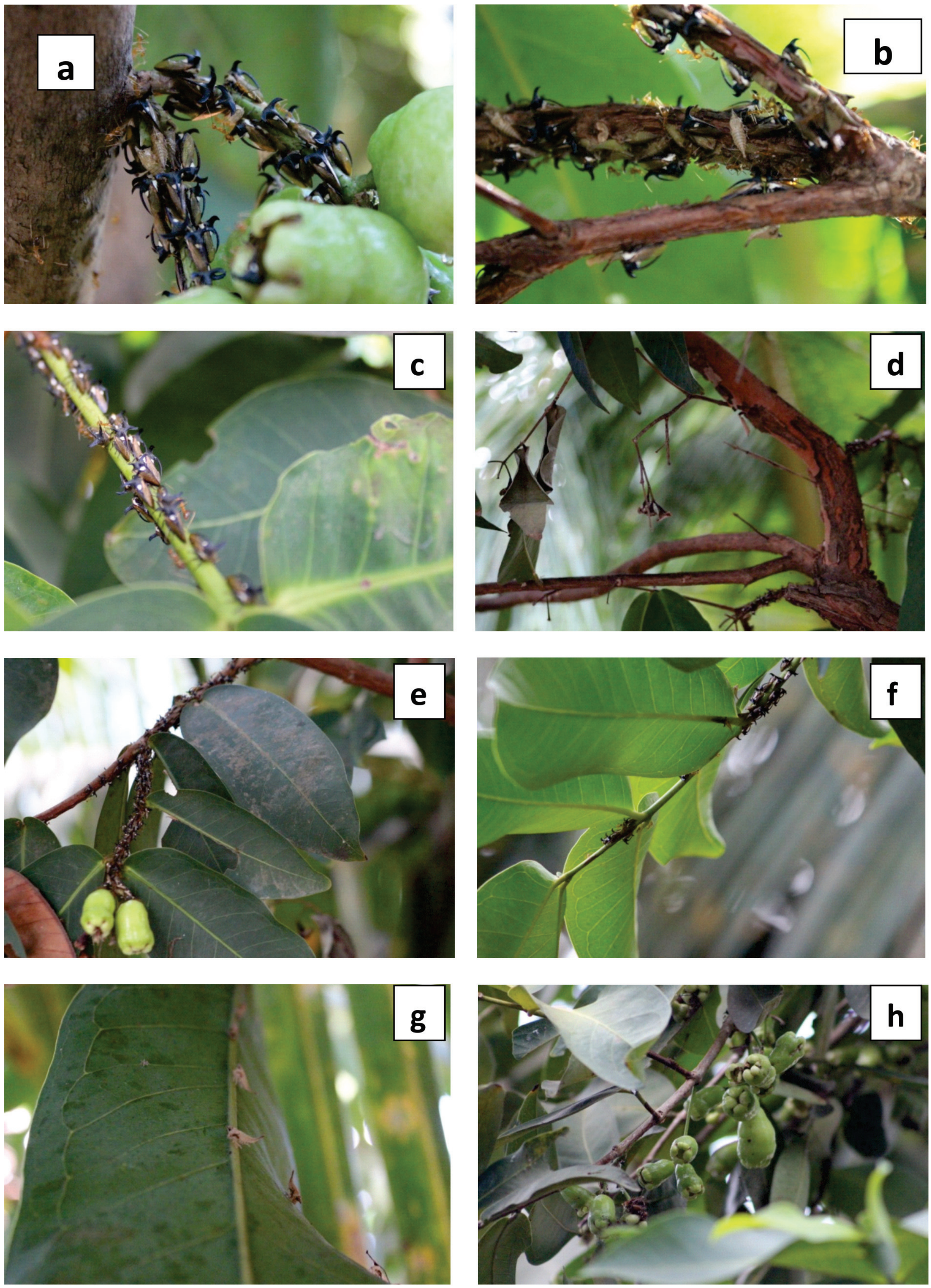

Fig. 1 Infestation of *Oxyrachis tarandus* on *Syzygium aqueum.* (a) Peduncle (PD), (b) Old Twig (OT), (c) Young Terminal Branch (YTB), (d) affected area showing wilting and scars, (e) Continuous patch of OT, YTB and PD, (f) Newly invading patch, (g) Exuvium on leaf and (h) Non-infested upper branches

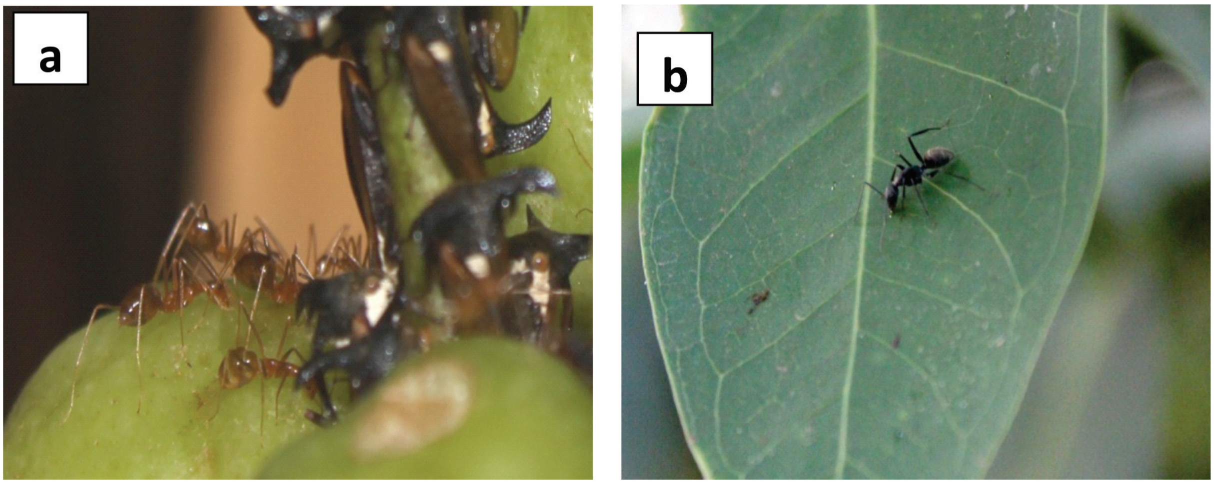

Fig. 2. Mutualistic ants *Oecophylla smaragdina* (a) attending cow bugs and *Camponotus compressus* (b) foraging to suck the honey dew from surfaces.

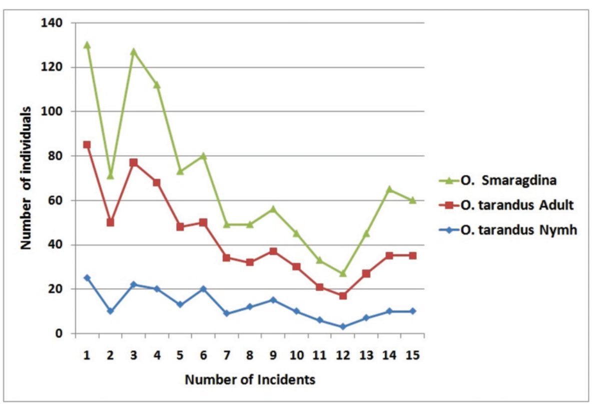

Fig. 3a. Graphs showing number of *Oxyrachis tarandus and Oecophylla smaragdina* observed in the infestation patches of *Syzygium aqueum*

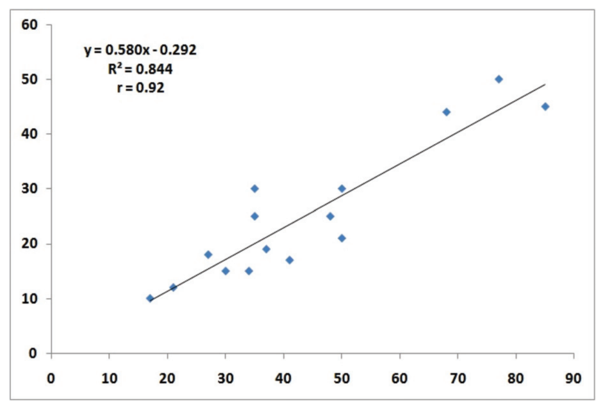

Fig. 3b. Correlation between number of *Oxyrachis tarandus and Oecophylla smaragdina* observed in the infestation patches of *Syzygium aqueum.*

peak period was 25°C and 35°C respectively. This is in line with the previous report on *O. tarandus* peak infestation on *Withania somnifera* (Ashwagandha) plant during March-April with average minimum and maximum temperature 16°C and 31°C respectively (Sharma and Patil, 2011) and on *Acacia nilotica* (Ali *et al*., 2008). Occurrence of *O. tarandus* shows a good positive correlation with environmental factors such as low temperature, high humidity and rainfall (Rahmathulla *et al*., 2015). Previous observations report peak infestation of *O. tarandus* also during November to January in pigeon pea (Claver, 2011) and June to July in mulberry (Rahmathulla *et al*., 2015). Variation in the peak of occurrence on different host across India at different months suggests host or climate specific change in the infestation period, but further studies are required to confirm it. Out of 81patches of infestation observed, 48 were PD patches (Fig. 1a), 20 YTB (Fig. 1c) and 13 OT (Fig. 1b). The cow bug used a much wider range of shoot length classes than other members of the membracidae (Price and Carr, 2000). No patches were observed on MB and L. On the lower side of the leaf, at random, about 3-5 exuvium were observed (Fig. 1g). On an average 19.8 ± 5.3 adult and 7.2 ± 2.9 nymph cow bugs were present over a PD patch of 7.0 ± 2.4 cm length. This account for a population

density of 1.52 individuals per cm^2 patch area which is significantly higher ($P < 0.05$) than all other patches observed. PD patches were retained about a week even after the fruit fall off from the peduncle, but with gradual decrease in the number of individuals over time. Distance between adjacent PD patches was not uniform as it depends on how frequently fruit stalk is present over a branch. Previous reports of patchy distribution of *O. tarandus* on host plants discussed mainly on the YTB patches and observation on peduncle patches (PD) is for the first time. There observed 23.4 ± 4.2 adults and 13.2 ± 4.4 nymphs over a YTB patch of 12.8 ± 1.9 cm length. This accounted for a population density of 0.66 individuals per cm^2 patch area which is significantly higher ($P < 0.05$) than OT lower than PD patches. Lowest density, 0.16 individuals per cm^2 patch, was recorded at OT where 47.6 ± 10.3 adults and 18.0 ± 6.3 nymphs were present over OT patch of 18.3 ± 5.3 cm length. Five situations, where OT, YTB and PD patches was continuous without any empty area in between comprising a length of 49.8 ± 4 cm were also observed (Fig. 1e). Even though patches without nymphs are seen, nymphs were always accompanied by adults (Fig. 1a-c). Offspring survival rates in treehoppers are improved through maternal care which represents an important behavioral and life history modification in them. Early treehopper instar's stylet is not strong enough to penetrate the epidermis of host plant tissues to suck the sap. At this situation adult females modify branches by making series of feeding slits to ensure the food resources accessible to nymphs (Wood, 1993).

Oecophylla smaragdina F. (Fig. 2a) and *Camponotus compressus* F. (Hymenoptera: Formicidae) show mutualism with cow horn bug for its honey dew (Way, 1963; Way and Khoo, 1992; Renault *et al.*, 2005; Nettimi and Iyer, 2015). There was a strong positive correlation between the number of *O. tarandus* and *O. smaragdina* in infestation patches with a correlation coefficient of 0.92 (Fig. 3a, b). Nettimi and Iyer (2015) reported strong positive correlation between *C. compressus* and *O. tarandus* on *Bauhinia tomentosa*. *Camponotus compressus* (Fig. 2b) are also observed but very rarely in patches with a frequency of 0.01 (1/100 observations) and therefore direct interaction of them with *O. tarandus* is too less. Outer to patches around 20 ants/ observation were found running so fast and stopping to suck the honey dew whenever they encounter it over the surface including leaf. Their lesser chance for direct interaction with *O. tarandus* forces them to utilize the minimum available honey dew, left by *O. smaragdina,* with minimum number of individuals. Abundance of *O. smaragdina* population check the number *C. compressus* in different host ranges as reported earlier by Ranga rao and Shanower (1999) and Sharma and Sundararaj (2011). Infestation of *O. tarandus* caused wilting and defoliation, but not at high rate. The size of fruit on infested and non infested peduncle shows significant difference, the infested fruit being shrinked and with pointed brown spot in large number. *O. tarandus* was recorded on mulberry plant, *Morus alba* (Sunil *et al*., 2003; Avhad and Hiware, 2013) and on sapling of *Dalbergia sissoo* (Sah and Ali, 2005). The review of literature reveals that this is first report of *O. tarandus* infestation on *S. aqueum* and as new host plant.

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Morphological and biochemical traits in chickpea resistance against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

Manish K. Yadav*, Lipsa Das, V. Ramalakshmi, Nihal R. Sunil, Sudhanshu Bala Nayak and Deepayan Padhi

Department of Agricultural Entomology, M. S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi 761200, Odisha, India
Email: manish.yadav@cutm.ac.in; arpansfour@gmail.com

ABSTRACT: Investigation undertaken with eight chickpea genotypes (Vallabh Kallar Channa 1, Ankur (CSJ 140), JGK-2, Ganguar (GNG 1581), Jawahar Gram-1 (JGK 1), WCG-10 (Pant G-10), Avrodhi and ICC 506-EB as resistant check) for their morphological and biochemical traits of resistance to *Helicoverpa armigera,* indicated trichome number, length and density, and nitrogen, total chlorophyll and potassium content as influencing the resistance/ susceptibility levels in chick pea.

KEYWORDS: Pod borer, trichome, chlorophyll

Chickpea *(Cicer arietinum* L.), which is commonly known as gram plays an important role in the vegetarian diet as a major source of protein. Pulses are almost an essential component of the vegetarian diet in Indian sub-continent, besides in rich source of protein. Insect-pests are challenge in gram cultivation. NCIPM study has recorded pod borer, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in gram to cause 30-90% loss in Madhya Pradesh, Karnataka, Rajasthan, Maharashtra, Uttar Pradesh, Gujarat and Telangana (Kumar *et al*., 2021a, b; Shah *et al*., 2021). This polyphagous pest cause economic loss to chickpea, pigeonpea, cotton, sunflower, tomatoes, chillies, tobacco and many other crops (Vikram, 2021). Among several insect pests attacking chickpea, gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is the major pest of chickpea which feeds on every stage of the crop from seedling to maturity and is known to cause 50 – 60 per cent pod loss (Sonawane and Chaudhary, 2021). Morphological namely plant height, stem thickness, number of branches, leaf trichome density and trichome length and biochemicals namely nitrogen, protein, potassium, phosphorus and total chlorophyll contents are the important traits in contributing resistance or otherwise. Therefore, the present investigation was undertaken to calibrate the influence of various traits and their impact on gram pod borer infestation on chick pea.

Experiment was conducted during the *Rabi* 2020-2021 with a total number of eight genotypes (Table 1). The ICC 506-EB genotype was selected as

* Author for correspondence

Table 1. Morphological traits, biochemical contents of the chickpea genotypes and their *Helicoverpa armigera* infestation

Genotypes	Morphological characters					Biochemical content				*Helicoverpa armigera* infestation		
	Plant heigh (cm)	Stem thickness (mm)	Branches (no.)	Trichome density ($leaf^{1}$)	Trichome length (ìm)	N (%)	Protein (%)	K (%)	Chlorophyll (mg/ml)	Larvae/ plant	Leaf (%)	Pod (%)
Vallabh Kallar Channa 1	54.34	3.67	5.67	43.61	7.34	3.61	22.56	0.91	1.51	3.67	67	61
Ankur (CSJ 140)	52.67	4.34	6.34	47.00	7.11	3.36	21.00	0.94	1.46	2.34	46	51
Avrodhi	56.34	4.34	5.34	48.34	6.67	3.19	19.93	1.01	1.44	2.00	39	41
Ganguar (GNG 1581)	54.67	4.67	4.67	47.67	7.18	3.34	20.87	0.96	1.46	2.34	48	48
Jawahar Gram-1 (JGK 1)	52.34	4.00	6.00	49.34	7.34	3.21	20.06	0.97	1.42	1.67	37	41
WCG-10 (Pant G-10)	56.67	3.67	5.67	46.67	7.67	3.41	21.31	0.91	1.39	2.67	51	53
JGK-2	54.34	4.34	5.34	41.67	6.91	3.47	21.68	0.92	1.53	3.34	59	53
ICC 506-EB (Check)	51.34	4.34	5.34	51.34	6.41	3.09	19.31	1.03	1.37	1.34	36	38
Sem±	0.73	0.36	0.43	0.95	0.54	0.36	0.43	0.11	0.19	0.39	0.8	0.9
CD @0.05%	2.14	1.03	1.21	2.81	1.54	0.93	1.21	0.29	0.49	1.09	2.3	2.7
CV	3.68	2.51	2.66	3.43	2.37	2.31	1.63	1.09	1.21	2.17	3.6	3.5

resistant check as it showed resistance against *H. armigera* on the basis of Malic acid content in leaves (Bhagwat *et al*., 1995). CRBD has been utilized with all the recommended agronomic practices, sown in rows (10 X 35 cm) with three replications. Number of larvae per plant, mean leaf infestation and mean pod infestation were recorded in all the replications by selecting five random plants at the time of pod formation stage. Morphological parameters viz., plant height, stem thickness, number of branches, leaf trichome density and trichome length were recorded. For trichome extraction, leaf chlorophyll was erased with the help of organic solvents (dimethyl sulpho-oxide and ethanol) kept for 12 hours at 60^0C in BOD. Later on, the trichomes were extracted with help of fine forceps, needle and surgical blades and length has been taken by placing over the measuring ocular under the microscope.

For the estimation of biochemical (nitrogen, phosphorus, potassium and protein) samples have been prepared by selecting a total number of five plants from each replication of each treatment randomly and cut from the base at root. These samples should be composited, washed with running water and then chopped into very small pieces by mixing stem and leaves at the rate of 80:20. Later on, the chopped material was mixed thoroughly and dried in air for 1 day and later in the oven at 70^0C for a total period of 22-28 hours. These dried samples were again ground upto finer dust. From these, 5 g material was taken for estimation of N, K and protein. Nitrogen content was calculated as per AOAC (1970).

$$\text{Nitrogen (\%)} = \frac{\text{(Sample TV-Blank TV) } 0.00007 \times \text{Volume of digation}}{\text{weight of sample} \times \text{Adiquot taken}} \times 100$$

The protein calculated as = Nitrogen (%) × 6.25; potassium by Flame Photometer (Upadhyay and Sahu, 2012) and total chlorophyll by spectrophotometer method (Arnon, 1949). One gram leaf samples were taken, homogenized in a pre-cooled mortar and pestle using 80 per cent acetone. A pinch of calcium carbonate was added while grinding. Later the extract was centrifuged at 3000 rpm for 15 min and made up to 25 ml with 80 per cent acetone. The clear solutions were transferred to a colorimeter tube and the optical density was measured at 645 nm and 663 nm, against an 80 per cent acetone blank in Shimadzu 35 Double Beam spectrophotometer (UV 240). The levels of chlorophyll 'a' and chlorophyll 'b' were determined using the equation given below:

$$\text{Chlorophyll-a [mg ml}^{-1}] = 12.7\ A_{663} - 2.69\ A_{645}$$

$$\text{Amount of Chlorophyll-b [mg ml}^{-1}] = 22.9\ A_{645} - 4.68\ A_{663}$$

where:

A_{645} = absorbance at a wavelength of 645 nm

A_{663} = absorbance at a wavelength of 663 nm.

Total Chlorophyll (mg/ml) = Chlorophyll a + Chlorophyll b.

The data were analysed for ANOVA and correlation to establish the relationship of traits with the levels of pod borer infestation.

Plant heights ranged from 51.34 to 56.67cm and maximum height was recorded in WCG-10 (Pant G-10) followed by Avrodhi with 56.34 and Ganguar (GNG 1581) with 54.67cm while minimum was recorded in ICC 506-EB (Check) with 51.34. Stem thickness was maximum in Ganguar (GNG 1581) with 4.67 mm followed by Ankur (CSJ 140); JGK-2; Avrodhi and ICC 506-EB (Check) and minimum was recorded in WCG-10 (Pant G-10) and Vallabh Kallar Channa 1 with 3.67 mm. Number of branches were maximum in Ankur (CSJ 140) with 6.34 followed by Jawahar Gram-1 (JGK 1), Vallabh Kallar Channa 1 and WCG-10 (Pant G-10). Trichome density was maximum in the leaves of ICC 506-EB (Check) with 51.34 trichomes per leaf, followed by Jawahar Gram-1 (JGK 1). Trichome density was minimum in JGK-2 with 41.67 and Vallabh Kallar Channa 1 with 43.61. The trichome length was maximum in WCG-10 (Pant G-10) with 7.67 ìm followed by Vallabh Kallar Channa 1 and Jawahar Gram-1 (JGK 1) with 7.34 ìm. Minimum trichome length was recorded in ICC 506-EB (Check) with 6.41 µm and Avrodhi with 6.67 µm (Table 1).

Nitrogen content was maximum in Vallabh Kallar Channa 1 (3.61%) followed by JGK-2 and WCG-10 (Pant G-10), while minimum was recorded in ICC 506-EB (Check) (3.09 %) and Avrodhi (3.19%). In the case of protein, it was maximum in Vallabh Kallar Channa 1 (22.56 %), followed by JGK-2 (21.68%) and WCG-10 (Pant G-10) (21.31%). Potassium was maximum in ICC 506-EB (Check), while minimum was in Vallabh Kallar Channa 1 and WCG-10 (Pant G-10). The total chlorophyll was maximum in JGK-2 (1.53 mg ml^{-1}) followed by Vallabh Kallar Channa 1 and Ankur (CSJ 140); Ganguar (GNG 1581), while it was minimum in ICC 506-EB (Check) (1.37 mg ml^{-1}) (Table 1).

The number of pod borer larvae was maximum in Vallabh Kallar Channa 1 (3.67) followed by JGK-2 and WCG-10 (Pant G-10), while minimum in ICC 506-EB (Check) (1.34). The infestation on leaf was maximum in Vallabh Kallar Channa 1 (67%) followed by JGK-2 (59%) and WCG-10 (Pant G-10) (51 %), while it was minimum in ICC 506-EB (Check) (36%). The mean per cent of pod infestation (Table 1) was found maximum in Vallabh Kallar Channa 1 (61%) followed by WCG-10 (Pant G-10); JGK-2 (53%) and Ankur (CSJ 140) (51%). It was minimum in ICC 506-EB (Check) (38%).

Significant positive correlation was noted between trichome length, total chlorophyll, nitrogen and pod infestation (Table 2). Plant height is an important character of plant, in case of all the agricultural crops, it deals with the productivity of the crop. All the selected genotypes under this experiment had showed different degree of variation among the height parameter. As per the correlation values, height is found to be positively correlated with the various infestation parameters. Dinesh *et al*. (2017), Pandey *et al*. (2021) and Yadav *et al*. (2021) support these findings. The stem thickness can be considered as a major factor in case of stem borers but the defoliators have not found to be correlated with the stem thickness that much. In case of other morphological parameters, trichome density and trichome length was found to the major infestation governing factor in case of this pest. The correlation of trichome density with various traits of infestation was maximum and negative and it was noted that genotypes having shorter trichomes with higher density, was found to be very less infested with the chickpea pod borer (Shahzad *et al*., 2005; Vanambathina *et al*., 2021).

Nitrogen, potassium and chlorophyll were noted as the more influencing factors to the infestation and survival of chickpea pod borer on selected genotypes. The maximum correlation was seen in case of mean per cent of pod infestation and nitrogen content in plants. The correlation between nitrogen; chlorophyll and infestation traits suggested the more nitrogen content in plants will leads to more infestation of *H. armigera* (Gyawali *et al*.,

Table 2. Correlation between the morphological and biochemical factors of chickpea genotypes and *Helicoverpa armigera* infestation

Character	Larvae/ plant	Leaf infestation (%)	Pod infestation (%)	Correlation
Plant height	0.439	0.346	0.361	Positive
Stem thickness	-0.390	-0.407	-0.478	Negative
Branches (no.)	-0.010	-0.046	0.142	NS
Trichome density	-0.952	-0.904	-0.840	Negative
Trichome length	0.449	0.440	0.604	Positive
Nitrogen	0.972	0.922	0.987	Positive
Potassium	-0.852	-0.834	-0.918	Negative
Chlorophyll	0.804	0.758	0.665	Positive

2021; War *et al.*, 2021). The potassium found to be negatively correlated with all traits of infestation and genotypes richer in K content, were slightly resistant against the chickpea pod borer infestation (Keshan *et al*., 2021; Gayatri and Kumar, 2021; Sai *et al*., 2021**).** The results indicate trichome density and lengths as the major influencing factors while in biochemical, nitrogen, potassium and chlorophyll content were identified in influencing the susceptibility levels of the genotypes.

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Diversity of ants (Hymenoptera: Formicidae) in the University of Kerala Campus, Thiruvananthapuram, India

Anupa K. Antony, Merin Elizabeth George and G. Prasad*

*Department of Zoology, University of Kerala, Kariavattom, Thiruvananthapuram 695581, Kerala, India. Email: shininglantern90@gmail.com;megkattimattom@gmail.com; probios1@gmail.com**

ABSTRACT: Survey conducted on the ant diversity in the Kerala University Campus revealed a total 64 species under six subfamilies. Species belonging to the Myrmicinae dominated (51.5%) followed by Formicinae (20.6%), Ponerinae (13.2%), Dolichoderinae (4.4%), Pseudomyrmicinae (4.4%) and Dorylinae (1.5%). Endemic species *Camponotus invidus* Forel, 1892 , *Cardiocondyla parvinoda* Forel, 1902, *Carebara spinata* Bharti & Kumar, 2013 and *Tetramorium rossi* (Bolton, 1976) were recorded in the campus. *Anoplolepis gracilipes* (Smith, 1857), *Paratrechina longicornis* (Latreille, 1802), *Monomorium carbonarium* Smith 1858, *Solenopsis geminate* (Fabricius, 1804), *Strumigenys membranifera* Emery, 1869, *Tetramorium bicarinatum* (Nylander, 1846) and *Hypoponera ragusai* (Emery, 1894) (introduced species) were found in the campus. The results showed that the campus is rich in ant diversity. The sites with human interference showed less diversity. A potential new species in the genus *Lepisiota* was recorded. *Trichomyrmex abberans, Carebara spinata, Crematogaster anthracina, Crematogaster biroi* and *Nylanderia indica* are new records.

KEYWORDS: Formicidae, subfamilies, Shannon-Wiener diversity index, Shannon Evenness Index, Margalef's index

Ants are one of the most important among insects in terms of their contribution to the ecosystem. They can function as ecosystem engineers by changing the chemical and microbial properties of the soil they occupy (Holec and Frouz, 2006). Ant mounds have been shown to increase the nitrates and phosphorus in the soil (Nkem *et al.*, 2000) and act as indicators of soil microbial biomass restoration (Andersen and Sparling, 1997). In addition to changing soil properties, ants also help in seed dispersal (Gammans *et al.*, 2005). They are important predators both in forests (Philpott and Armbrecht, 2006) and in agro-ecosystems (Mollot *et al.*, 2012). Ants have been reported as biocontrol agents in banana (Abera-Kalibata *et al.*, 2008; Mollot *et al.*, 2012; Wang *et al.*, 2016), in mango and citrus (Offenberg *et al.*, 2013; Thurman *et al.,* 2019). This is one of the main reasons they have been shown to increase crop yield (Offenberg and Wiwatwitaya, 2010; Evans *et al.*, 2011) Ant species diversity can be used as indicators to environmental changes (Tiede *et al*. 2017). Microclimatic changes can cause ant diversity to change and this can be used in bio-monitoring (Perfecto and Vandermeer, 1996). Ants are well understood, easy to sample and have a high biomass and diversity, which

* Author for correspondence

strengthen the logic in using them as bio-monitoring tools. Ant diversity indices can be used much better as indicators compared to many other taxa (Osborn *et al.*, 1999).

The present study aims to understand the ant diversity in the University of Kerala Campus, Kariavattom, Thiruvananthapuram. There have been studies on ant diversity in campuses across India previously by many authors (Ramesh *et al.*, 2009; Yashavantakumar *et al.*, 2016; Begum and Sandeep, 2018; Khan, 2018). This is the first comprehensive study of ants in the campus at Kerala University.

The survey was conducted for a period of three years from 2017 to 2019. Habitats were selected from the University of Kerala Campus (08^0 33'52.2"N and 076^0 53'14.8"E, elevation 53m above MSL). The entire campus, around 350 acres of land, divided into north and south regions were selected for the survey. From the north side, site 1 (Botanical garden), site 2 (mixed vegetation with bushy plants) and site 3 (woody plantation) were selected. From south region, site 4 (fruit trees predominantly sapota), site 5 (monoculture *Acacia* plantation), site 6 (mixed vegetation), and site 7 (bank area of a freshwater pond) were selected (Plate 1).

In each site five quadrants each with an area of 20x20 m^2 were marked and secured from human intervention. Methods used for collecting the ants include, litter sifting, beating low vegetation, and pitfall trap. Litter was collected from 1x1 m^2 quadrats. Hand picking was also done to ensure complete coverage of the sites. Ants collected were preserved (in 70% alcohol) immediately after collection (Agosti *et al.,* 2000). Identification of ants were done as per the keys (Bingham, 1903; Bolton, 1994; Bharti and Kumar 2012; Bharti and Wachkoo 2013a, b; Bharti *et al*., 2013, 2016; Bharti and Akbar 2014a, b). The specimens were processed, labeled and deposited in the museum of the Department of Zoology, University of Kerala. Photographic records of the specimens were taken for future reference. A checklist of all the species collected within the campus was prepared. The diversity indices (Shannon-Wiener diversity index, Shannon Evenness Index and Margalef's index) for the seven different habitats were calculated using the statistical software PAST, 2005.

A total of 710 ants were collected from the seven selected sites, comprising 64 species of ants belonging to six subfamilies viz., Dolichoderinae, Formicinae, Myrmicinae, Ponerinae, Pseudomyrmicinae and Dorylinae (Table 1). Maximum number of species recoerded was in the subfamily Myrmicinae (51.5%), followed by Formicinae (20.6%), Ponerinae (13.2%), Dolichoderinae (4.4%), Pseudomyrmicinae (4.4%) and Dorylinae (1.5%). The number of individuals collected was highest in Formicinae with 187 (52.8%), followed by Myrmicinae (122). Myrmicinae subfamily was more species rich with 34 species.

The presence/absence of ants recorded in the different sites is given in Table 1. Site 2 was more speciose with 43 species, while sites 5 and 7 showed lower species number, 14 and 19 species respectively. Site 1, 2 and 3 in north campus were more diverse (Table 2). The low species indices' rate in the south campus sites 4, 5, 6 and 7 could be due to high human interference. The south campus had more human intervention because most of the area was covered with buildings with little vegetation. Site 5 being a monoculture plantation was one of the reasons for the low diversity index (1.887). Monoculture plantations had low diversity because there were few diverse sources of habitat and food. During the study, there was construction work going on in the area near site 7. Anthropogenic factors like human interference and habitat fragmentation may have been the cause of lowered species diversity (Floren *et al*., 2001;Walter *et al.,* 2018; Martello *et al.,* 2018). This could explain the lowered species index (1.762) in site 7. Site 1 and 6 had more evenly distributed diversity indices (0.5328 and 0.5265 respectively). Site 1 was a botanical garden with a large variety of plants while site 6 had mixed vegetation. The diversity of the habitats in the area reflected in the species distribution. Overall, the site 1 Botanical garden was the most even and diverse site in this study. This shows that an area with natural diverse habitats

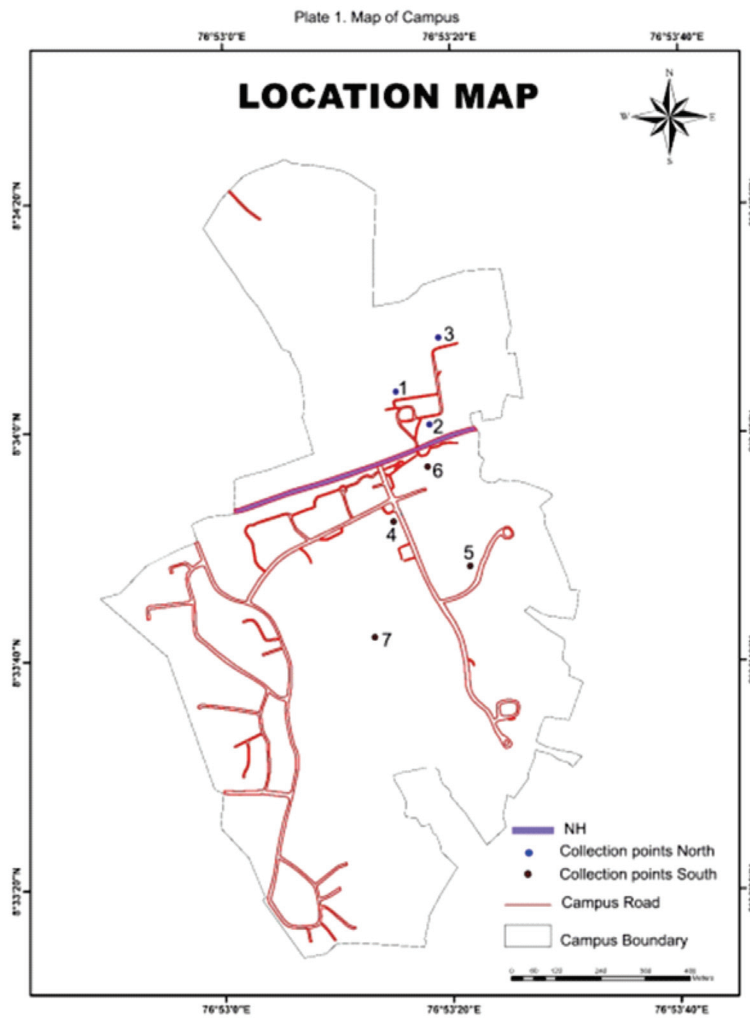

Fig. 1 Map of University of Kerala Campus

can ensure a better diversity for the region. Site 7 was the least diverse site showing that human interference is indeed unhealthy for the ecosystem (Bestelmeyer and Wiens, 1996). The north campus has only three buildings and is covered mostly with dense undisturbed vegetation. It also has a sacred groove which shows that the area is an undisturbed habitat. Consequently, we can find that the species richness is higher as well. The undisturbed habitat has ensured that the species diversity is higher (Walter *et al.,* 2018).When compared with previous studies on ant diversity in campuses, it can be seen that the University of Kerala campus has higher diversity (Yashavantakumar *et al.*, 2016; Ugare *et al.*, 2019).This shows that even though it is an urban area the region is ecologically important.

Anoplolepis gracilipes (Smith, 1857), *Camponotus compressus* (Fabricius, 1787), *C. invidus* Forel, 1892, *C. parius* Emery, 1889, *C. rufoglaucus* (Jerdon, 1851), *Oecophylla smaragdina* (Fabricius, 1775), *Meranoplus bicolor* (Guerin-Meneville, 1844), *Monomorium floricola* (Jerdon, 1851), *Diacamma rugosum* (Le Guillou, 1842) and *Odontomachus simillimus* Smith, 1858 were present in almost all sites. *Anoplolepis gracilipes* is an invasive species which can explain its presence in all sites (Holway *et al.,* 2002). *Odontomachus simillmus* is known to inhabit in

Plate 2. Ants

New Record in Kerala

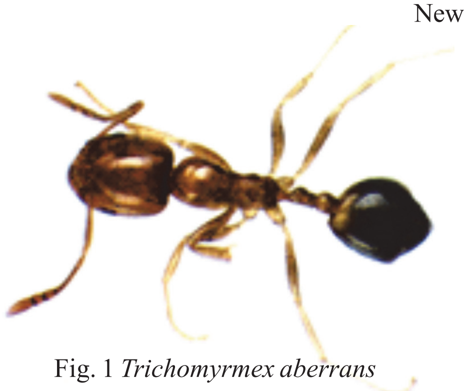

Fig. 1 *Trichomyrmex aberrans*

Fig. 2 *Nylanderia indica*

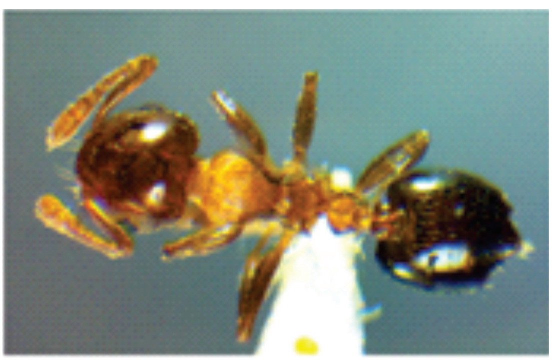

Fig. 3 *Crematogaster anthracina*

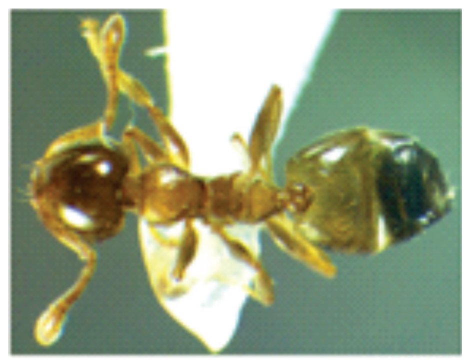

Fig. 4 *Crematogaster biroi*

Fig. 5 *Tetramorium bicarinatum*

;

disturbed areas. *Camponotus* spp. and *D. rugosum* are more generalist feeders and have more resilience to habitat disturbances (Abe and Uezu, 1977). *Plagiolepis jerdonii* Forel, 1894, *Polyrhachis tibialis* Smith, 1858, *Cardiocondyla parvinoda* Forel, 1902, *Carebara spinata* Bharti & Kumar, 2013, *Crematogaster anthracina* Smith, 1857, *C. dohrni* Mayr, 1879, *Monomorium carbonarium* Smith 1858, *Pheidole constanciae* Forel, 1902, *P. peguensis* Emery, 1895, *Strumigenys membranifera* Emery, 1869, *Trichomyrmex abberans* (Forel, 1902), *T. glaber* (Andre, 1883), *Hypoponera confinis* (Roger, 1860), *H. ragusai* (Emery, 1894), *Parvaponera darwinii* (Forel, 1893), *Platythyrea parallela* (Smith, 1859), *Tetraponera aitkenii* (Forel, 1902), *T. allaborans* (Walker, 1859) and *Cerapachys sp.* were found only in one site. Most of these ants

Table 1. Checklist of ants at University of Kerala and their presence in different sites with indication of species endemic (E) and indigenous to India (I)

Sl. No	Subfamily/ Scientific name	Sites
	Dolichoderinae	
1.	*Tapinoma indicum* Forel, 1895	2, 3
2.	*Tapinoma melanocephalum* (Fabricius, 1793)	1, 2, 6, 7
3.	*Technomyrmex albipes* (Smith, 1861)	1, 4
	Formicinae	
4.	*Anoplolepis gracilipies* (Smith, 1857) —— (I)	1, 2, 3, 4, 5, 6,7
5.	*Camponotus compressus* (Fabricius, 1787)	1, 2, 3, 4, 5, 7
6.	*Camponotus invidus* Forel, 1892 —— (E)	1, 2, 7
7.	*Camponotus irritans* (Smith, 1857)	2, 3
8.	*Camponotus parius* Emery, 1889	1, 2, 4, 5, 6, 7
9.	*Camponotus rufoglaucus* (Jerdon, 1851)	1, 2, 3, 4, 5, 6,7
10.	*Camponotus sericeus* (Fabricius, 1798)	1, 2, 3, 4, 7
11.	*Camponotus sp.* Mayr, 1861	2
12.	*Lepisiota sp.* Santschi, 1926	1
13.	*Nylanderia indica* (Forel, 1894)	6, 7
14.	*Oecophylla smaragdina* (Fabricius, 1775)	1, 2, 3, 4, 5, 6,7
15.	*Paratrechina longicornis* (Latreille, 1802) —— (I)	1, 3, 6, 7
16.	*Plagiolepis jerdonii* Forel, 1894	2
17.	*Polyrhachis exercita* (Walker, 1859)	1, 2, 3, 5
18.	*Polyrhachis scissa* (Roger, 1862)	1, 6
19.	*Polyrhahis thrinax* Roger, 1863	2, 3, 6, 7
20.	*Polyrhachis tibialis* Smith, 1858	4
	Myrmicinae	
21.	*Cardiocondyla parvinoda* Forel, 1902 —— (E)	1
22.	*Cardiocondyla wroughtonii* (Forel, 1890)	3, 6, 7
23.	*Carebara spinata* Bharti & Kumar, 2013 —— (E)	2
24.	*Crematogaster anthracina* Smith, 1857	5
25.	*Crematogaster biroi* Mayr, 1897	1, 2, 3
26.	*Crematogaster dohrni* Mayr, 1879	2
27.	*Crematogaster flava* Forel, 1886	1, 2
28.	*Crematogaster rothneyi* Mayr, 1879	1, 2, 3, 4, 7
29.	*Lophomyrmex quadrispinosus* (Jerdon, 1851)	2, 6
30.	*Meranoplus bicolor* (Guerin-Meneville, 1844)	1, 2, 4, 5, 6,7
31.	*Messor himalayanus* (Forel, 1902)	3, 5
32.	*Monomorium bicolor* (Bolton, 1987)	1, 4, 5
33.	*Monomorium carbonarium* Smith 1858 —— (I)	2
34.	*Monomorium floricola* (Jerdon, 1851)	1, 2, 4, 5, 6,7
35.	*Monomorium orientale* Mayr, 1879	1, 2
36.	*Pheidole constanciae* Forel, 1902	4
37.	*Pheidole peguensis* Emery, 1895	4
38.	*Pheidole* sp. 1 Westwood, 1839	3
39.	*Pheidole* sp. 2 Westwood, 1839	3
40.	*Solenopsis geminata* (Fabricius, 1804) —— (I)	2, 7
41.	*Strumigenys membranifera* Emery, 1869 —— (I)	2
42.	*Strumigenys aduncomala* De Andrade, 2007 —— (E)	2, 3
43.	*Tetramorium bicarinatum* (Nylander, 1846) ——- (I)	1, 2, 3
44.	*Tetramorium inglebyi* Forel, 1902	1, 2, 3, 4
45.	*Tetramorium lanuginosum* (Mayr, 1870)	1, 3
46.	*Tetramorium obesum* Andre, 1887	1, 2
47.	*Tetramorium rossi* (Bolton, 1976) —— (E)	1, 2, 4
48.	*Tetramorium walshi* (Forel, 1890)	1, 3, 6
49.	*Tetramorium smithi* Mayr, 1879	3, 4
50.	*Trichomyrmex abberans* (Forel, 1902)	3

Sl. No	Subfamily/ Scientific name	Sites	Sl. No	Subfamily/ Scientific name	Sites
51.	*Trichomyrmex glaber* (Andre, 1883)	1		Smith, 1858	1, 2, 4, 5, 6
	Ponerinae		59.	*Parvaponera darwinii* (Forel, 1893)	2
52.	*Anochetus graeffei* Mayr, 1870	1, 2, 3, 6	60.	*Platythyrea parallela* (Smith, 1859)	4
53.	*Brachyponera jerdonii* (Forel, 1900)	1, 2, 3, 6		Pseudomyrmecinae	
54.	*Diacamma rugosum* (Le Guillou, 1842)	1, 2, 3, 4, 5, 6, 7	61.	*Tetraponera aitkenii* (Forel, 1902)	2
55.	*Hypoponera confinis* (Roger, 1860)	6	62.	*Tetraponera allaborans* (Walker, 1859)	2
56.	*Hypoponera ragusai* (Emery, 1894) —— (I)	1	63.	*Tetraponera nigra* (Jerdon, 1851)	1, 3
57.	*Leptogenys peuqueti* (Andre, 1887)	1, 2, 3, 7		Dorylinae	
58.	*Odontomachus simillimus*		64.	*Cerapachys sp.* Smith, F., 1857	2

(E) - Species endemic to India; (I) - Species indigenous to India

Table 2. Diversity indices in the different sites of campus

Site	No. of species	Shannon-Wiener	Shannon Evenness	Margalef's Index
1	37	2.981	0.5328	6.146
2	43	2.63	0.3302	4.407
3	29	2.574	0.4858	6.796
4	20	2.232	0.4657	4.288
5	14	1.887	0.4712	3.215
6	20	2.254	0.5265	4.212
7	19	1.762	0.3066	3.722
North Campus	61	3.207	0.4752	8.593
South Campus	32	2.66	0.4085	5.782

were found in only site 2 which perhaps due to the site being mixed vegetation as it provides more microhabitats for different ants.

In addition to the high species diversity five species, *Trichomyrmex abberans, Carebara spinata, Crematogaster anthracina, C. biroi,* and *Nylanderia indica* were found as new records from Kerala (Figs. 1-5) and the specimens were deposited at Department of Zoology, University of Kerala, Kariavattom. The first records were published as two papers (Antony *et al.*, 2018; Antony and Prasad, 2019) (Figs. 6-8). *A potential new species belonging to the genus, Lepisiota* was also found and the species is yet to be identified. These range extensions were identified using Bharti *et al.* (2016). *Camponotus invidus, Cardiocondyla parvinoda, Carebara spinata,* and *Tetramorium rossi* are species endemic to India found in the campus. *Anoplolepis gracilepis, Paratrechina longicornis, Monomorium carbonarium, Solenopsis geminata, Strumigenys membranifera, Tetramorium bicarinatum,* and *Hypoponera ragusai* are introduced species found in the campus.

The present study shows that the University of Kerala Campus, Kariavattom, is highly species rich with numerous endemic species of ants. The diversity patterns found in the study are similar to that found in previous studies where human interference showed a lowered diversity. The campus diversity must be preserved to ensure the better conservation of ant species. The results also show that ant diversity can be used to understand the anthropogenic impact on forested areas.

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Report of *Neoheterophrictus chimminiensis* Sunil Jose, 2020 (Araneae: Theraphosidae) from the Nelliyampathy forest region of Western Ghats, India

K. Karthika* and K. Sunil Jose

Arachnology Lab, Department of Zoology, Deva Matha College, Kuravilangad, 686633, Kerala, India. Email: krkkarthikak@gmail.com; sunil32@gmail.com

ABSTRACT: *Neoheterophrictus chimminiensis* Sunil Jose, 2020 was previously only found in the Chimmini forest area, but it has recently been found in the Nelliyampathy forest region of Western Ghats, indicating its distribution. Taxonomic description illustrations and measurements of *N. chimminiensis* are added.

KEY WORDS: Endemic theraphosid, distribution, tarantula, mygalomorph, rastellum

Neoheterophrictus includes *N. amboli* Mirza & Sanap, 2014, *N. bhori* Gravely, 1915, *N. crurofulvus* Siliwal, Gupta & Raven, 2012, *N. madraspatanus* Gravely, 1935, *N. sahyadri* Siliwal, Gupta & Raven, 2012, *N. smithi* Mirza, Bhosale & Sanap, 2014, *N. uttarakannada* Siliwal, Gupta & Raven and *N. chimminiensis* Sunil Jose, 2020 (Gravely, 1915; Siliwal *et al*., 2007, 2012; Mirza *et al.,* 2014; Sunil Jose, 2020; World Spider Catalog, 2021). Recently Sunil Jose (2020) reported *N. chimminiensis* its occurrence from Chimmini wildlife Sanctuary of Kerala. The majority of current records on the distribution of this genus come from Karnataka, with only a few records of its presence in Kerala.

During the field trips *N. chimminiensis* was observed in the Nelliyampathy forest range of Western Ghats in Kerala. The specimens collected in 70 per cent ethyl alcohol were deposited in the Biodiversity Museum, Deva Matha College, Kuravilangad, Kerala. The whole body including legs and eye measurements and photographs were taken using LASX application suite X software. Spermathecae was cleared in clove oil. Leg measurements except claws were recorded. Measurement of chelicerae was taken after dissecting out separately. All measurements are in mm. Specimens are observed using in Leica Automontage stereozoom microscope attached with FLEXACAM1-C1 camera. Abbreviations used: AME - Anterior median eye; ALE - Anterior lateral eye; PME-Posterior median eye; PLE - Posterior lateral eye; ^{2} - ^{2}V- first Leg to fourth leg; mt – Metatarsus; ti - tibia, ta - tarsus, lt - lateral, v - ventral.

Neoheterophrictus chimminiensis Sunil Jose, 2020 (Figs. 1-4)

DMCK 13/135, Holotype, Coll. Sunil Jose in 2013, Chimmini Wildlife Sanctuary. Other material examined: DMCK 20/367, coll. Karthika K, Aswathy S and Linta Joseph, Nelliyampathy forest range.

* Author for correspondence

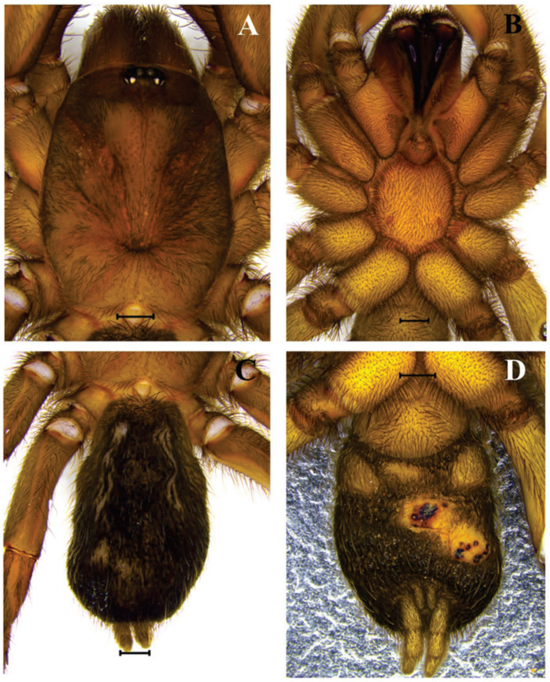

Fig. 1A. *N. Chimminiensis* Carapace dorsal view, B. Sternum, C. Abdomen dorsal view and D. Abdomen ventral view

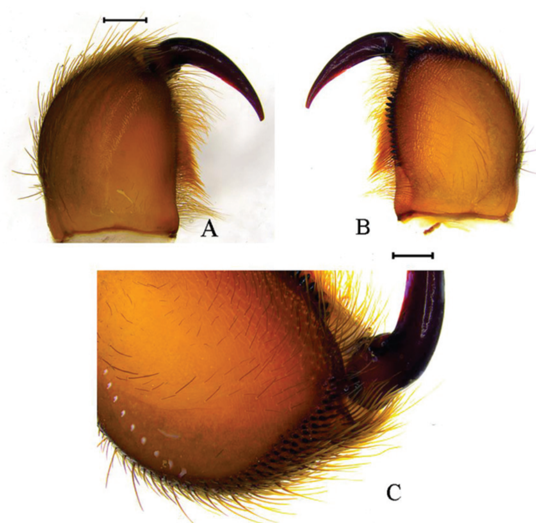

Fig. 2 *N. Chimminiensis* Chelicerae; Prolateral view, B. Retrolateral view, C. Antero-ventral view showing Rastellum

Diagnosis: Multilobed spermathecae with equally spaced four lobes present in *N. chimminiensis* differs from *N. bhori* having six spermathecal lobes and a straight coxal suture, while slightly curved suture present in *N. bhori.* Rastellum present on the anterodorsal chelicerae of *N. chimminiensis.*

Fig. 3A. Eye, B. Maxillae, labium, C. Coxae I retro-lateral view, D. Spinneret E. Spermatheca.

Fig. 4a. *N. chimminiensis* in their habitat

Fig. 4b. Collection site at Nelliyampathy forest range

Further it differs in the presence of stridulatory setae in prolateral side of coxae I.

Colour: The entire body is solid black, with light yellow long thin hairs interspersed across the abdomen. There are no distinct markings on the

abdomen, which is oval or circular in shape. Legs were thick black in colour.

Measurements (mm): Total length: 17.46, carapace length: 5.08 long and 4.10 wide, Abdomen: 12.38 long and 4.52 wide. Chelicerae: 3.97 long and 3.08 wide. Eyes: Interdistance: AME-AME 0.10, PLE-PME 0.07, ALE-PLE 0.12, AME-PME 0.12. Eye Diameter: AME 0.21, ALE: 0.14, PLE0.8 PME-0.13. Measurements of leg: I- 3.60, 1.64, 2.78, 1.43, 1.21; II- 2.75, 1.11, 2.23, 1.34, 1.34; III- 2.52, 1.76, 1.38, 2.03, 1.38; IV-3.56, 2.31, 2.77, 3.57, 1.79; Palp- 2.61, 1.04, 1.79, 1.54; Spinneret PME-0.78, PLE-3.61 long. PLE-Basal segment- 1.39, Median segment - 0.95, Posterior segment 1.27 long.

Description: Female: Cephalothorax (Fig. 1 A) - Longer than wider, plain Caput. Fovea is slightly procurved. Brown thoracic streaks arise from the fovea covered with hairs. Clypeus is absent or reduced. Eyes (Fig. 2 A) - ocular area - 1.79 wide and 1.00 length. AME is larger than rest. ALE is the smallest. Maxillae (Fig. 2 B) – with cupules (around 150) on the anterior triangular corners. Retrolateral face armed with Orangish yellow bush of hairs. Prolateral face covered with black hairs. Chelicerae (Fig. 3 A-C) -14 prolateral teeth. Retrolateral teeth much reduced or absent. Retrolateral face has no hairs. Rastellum present antero dorsally as small stout spines. Sternum (Fig. 1 B) - oval in shape, covered with black hairs. Anterior end is concave. Posterior end pointed separates the coxae ²V. Sigillae - Three pairs, posterior with a diameter of 0.18, median 0.14, anterior comparatively small, 0.07. Posterior sigillae are 0.65 away from median, sub central and median to anterior distance is 0.60, close to the margin. Leg: 4123, Tarsus and metatarsus of I and ²² is covered its ¼th with thin layer of scopulae while III and IV having scopulae covered the entire length of metatarsus and tarsus ventrally. In palp the tibia covers this thin layer of scopulae. Spines: No spines on the leg I. Leg II; ta-lt-2,v-1,mt-0, Leg III: mt-lt-4,v-6 ,ti-lt-2,v-3, Leg IV; ti-lt-2,v-4,mt-lt-4,v-6. Palp; ti- v-1.Coxae: Coxae ² (Fig. 2D) is characterised with suture on the retrolateral side. Presence of stridulatory spine below the suture. Coxae ²² – ²V stridulatory spines absent. Abdomen (Fig. 1 C-D) is black in colour somewhat oval in shape. Along pale yellow hairs intermixed with black hairs. Ventrally epigynal furrow is clear and paired book lungs. Spinneret (Fig. 2C) - two pairs. PLE larger than PME. Spermathecae (Fig. 3 E) - multilobed and transparent structures diverged to opposite side. Specifically four lobes attached to a stalk arising from the epigynal furrow.

The spider was found under a stone in a semi-evergreen forest (Figs. 4a-b). It attempted to hide beneath the rock in a little burrow-like hollow during capture. The spider kept in a terrarium (a habitat similar to their natural habitat, maintaining the temperature and moisture), was observed to feed on cockroach nymphs and crickets in captivity. Inside the terrarium, no burrowing behaviour was noted. The observation of *N. chimminiensis* in Nelliyampathy forest range adds to the range of extension of this species over Western Ghats of Kerala.

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Abrol, D.P., Sher-e-Kashmir University of Agricultural Sciences & Technology, Faculty of Agriculture, Main Campus Chatha, Jammu 180009, Jammu & Kashmir, India

Andrey B. Shatrov, Zoological Institute of the Russian Academy of Science, Universitetskaya nab., St Petersburg, Russia

Anil Kumar Dubey, Zoological Survey of India, Andaman and Nicobar Region Centre, Port Blair 744102, Andaman & Nicobar Islands, India

Anil Kumar Sethy, Institute of Wood Science & Technology, Institutes of Indian Council of Forestry Research & Education, Bangaluru 560003, Karnataka, India

Ankita Gupta, ICAR-National Bureau of Agricultural Insect Resources, H.A. Farm Post, Bellary Road, Hebbal, Bengaluru 560024, Karnataka, India

Ashish D. Tiple, Department of Zoology, Vidya Bharati college, Seloo, Wardha 442105, Maharashtra, India

Balakrishan, N., National Centre for Disease Control - Plague Surveillance Unit, Directorate General of Health Services, Ministry of Health & Family Welfare-Government of India, Bengaluru 560003, Karnataka, India

Balasubramanian, R., ICMR-National Institute of Virology-Kerala Unit, Dept. of Health Research, Min. of H&FW, Govt. T. D. Medical College Hospital, Vandanam, Alappuzha 688005, Kerala, India

Basker Parasuram, consultant Entomologist, National Centre for Disease Control, Directorate General of Health Services, Ministry of Health & Family Welfare-Government of India, Delhi, India

Belavadi, V.V., Department of Entomology, University of Agricultural Sciences, GKVK, Bangalore 560065, Karnataka, India

Bijoy, C., Research Department of Zoology, Christ College (Autonomous), Irinjalakuda, Thrissur 680125, Kerala, India

Chandish R. Ballal, ICAR-National Bureau of Agricultural Insect Resources, House No 460, 2nd Cross, 9th Main, HAL II Stage, Bangaluru 560008, Karnataka, India

Daniel Reegan, A., Regional Office for Health and Family Welfare, National Vector Borne Disease Control Program, Bangaluru, Karnataka, India

Debjani Dey, Division of Entomology, ICAR- IARI, New Delhi 110012, India

Devasahayam, S., ICAR - Indian Institute of Spices Research, Kozhikode 673012, Kerala, India

Farman ur Rahman Khan, Department of Biology; Deanship of Educational Services; Qassim University, Buraidah 51452, Al-Qassim, Kingdom of Saudi Arabia

George Mathew, International Forest Insect Pest Specialist (ADB Project), Forest Health Division, Kerala Forest Research Institute, Peechi 682013, Kerala, India

Hanamant Gadad, Central Tasar Research and Training Institute, Ranchi 835303, Jharkhand, India

Hebsi Bhai, Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Thiruvananthapuram 695522, Kerala, India

Hemant V. Ghate, Department of Zoology, Modern College, Shivaji Nagar, Pune 411005, Maharashtra, India

Himender Bharti, Ant Systematics and Molecular Biology Lab., Department of Zoology and Environmental Sciences, Punjabi University, Patiala 147002, Punjab. India

Hossein Lotfalizadeh, Plant Protection Research Department; East Azarbaijan Agricultural and Natural Resources Research & Education Center, AREEO, Tabriz, Iran

Jorge Ari Noriega, Department of Biogeography and Global change, Madrid, Spain

Josephrajkumar, A., ICAR-Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Kerala, India

Kamarasu, K., Institute of Vector control and Zoonoses, Directorate of Public Health and Preventive Medicine, Hosur 635126, Krishnagiri, Tamilnadu, India

Kesavan Subaharan, ICAR - National Bureau of Agricultural Insect Resources, Bangaluru 560064, Karnataka, India

Mahrad Nassirkhani, Entomology Department, Faculty of Agriculture and Natural Resources, Islamic Azad University, Arak branch, Arak, Iran

Mathew M. J., Dept. of Zoology, Sacred Heart College, Thevara, Kochi 682013, Kerala, India

Mohankumar, S., Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India

Mohd Yousuf, Tropical Forest Research Institute, Jabalpur 482021, Madhya Pradesh, India

Muhamed Jafer Palot, Zoological Survey of India, Western Regional Centre, Pune 411044, Maharashtra, India

Nair, K.S.S., Kerala Forest Research Institute, Peechi, Thrissur 680653, Kerala, India

Pandey, J.P., Silkworm Biotechnology laboratory, Central Tasar Research Training Institute, Ranchi ---, Jharkhand, India

Panicker, K.N., Amrita Institute of Medical Sciences, Kochi 682041, Kerala, India

Raghuraman, M., Professor, Department of Entomology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, UP, India

Rahmathulla, V.R. National Silkworm Seed Organization, Central Silk Board, Ring Road, Srirampura, Mysore, Karnataka 570008, India

Rajmohana, K., ENVIS Centre on Faunal Diversity, Zoological Survey of India, Kolkata 700053, West Bengal, India

Raman V., Vector Ecology and Management Unit, Department of Control of Neglected Tropical Diseases (CDS/NTD), World Health Organization, Geneva, Switzerland

Ramaraju, K., Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India

Ramasubramanian, T., ICAR-Sugarcane Breeding Institute, Coimbatore 641007, Tamil Nadu, India

Sabu K. Thomas, St. Joseph's College, Devagiri, Kozhikode 673008, Kerala, India

Sajan Jose K., Regal Bee Gardens Beekeeping Training Centre, Kanjar 685590, Idukki, Kerala, India

Sampath Kumar, M., Division of Insect Systematics, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru 560024, Karnataka, India

Sandeep Singh, All India Coordinated Research Project on Fruits, Department of Fruit Science, Punjab Agricultural University, Ludhiana 141004, Punjab, India

Santhosh Shreevihar, Department of Zoology, Malabar Christian College, Kozhikode 673001, Kerala, India

Sreekumar, K.M., College of Agriculture, Kerala Agricultural University, Padannakkad, Kasaragod 671328, Kerala, India

Srinivasa, N., Dept. of Entomology, University of Agricultural Sciences, GKVK, Bangaluru, 560065, Karnataka, India

Subramanian, K.A., Southern Regional Centre, Zoological Survey of India, Chennai 600028, Tamil Nadu, India

Sudhir Singh, Systematic Laboratory, Forest Entomology Division, Forest Research Institute, Dehradun 248006, Uttarakhand, India

Sundararaj, R., Forest Protection Division, Institute of Wood Science & Technology, Malleswaram, Bangaluru 560003, Karnataka, India

Sureshan, P.M., Western Ghat Field Research Centre, Zoological Survey of India, Eranhipalam, Kozhikode 673006, Kerala, India

Swaminathan, R., Department of Entomology, Rajasthan College of Agriculture, Maharnana Pratap University of Agriculture and Technology, Udaipur 313001, Rajasthan, India

Talmale, Zoological Survey of India, Western Regional Centre, Vidyanagar, Akurdi, Pune 411044, Maharashtra, India

Vasantharaj David, B., International Institute of Biotechnology & Toxicology, Padappai, Kancheepuram district, Tamil Nadu, India

Viraktamath, C.A., University of Agricultural Sciences, Bengaluru, Karnataka, India

Zeeshan A. Mirza, Post-Graduate Program in Wildlife Biology & Conservation, Wildlife Conservation Society-India Program, National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangaluru, India

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5. Dr. R.Viswanathan, Principal Scientist & Head, Division of Crop Protection, ICAR - Sugarcane Breeding Institute, Coimbatore 641007, Tamil Nadu

INFORMATION TO CONTRIBUTORS

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Department of Entomology, Kerala Agricultural University,
Vellayani PO, Thiruvananthapuram 695522, Kerala, India. E mail: aae@kau.in
web:www.entomon.in

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